HERBAL IMMUNE ENHANCERS AND INDIGENOUS HERBS, PLANTS AND FRUITS AND ITS TRADITIONAL IMPLICATIONS IN THERAPY INCLUDING ALTERNATIVE MEDICINES

Dr. Subha Ganguly (Editor-in-Chief)

ISBN: 978 – 978 – 52231 – 8 - 7

SCIENCE AND EDUCATION DEVELOPMENT INSTITUTE, NIGERIA

HERBAL IMMUNE ENHANCERS AND INDIGENOUS HERBS, PLANTS AND FRUITS AND ITS TRADITIONAL IMPLICATIONS IN THERAPY INCLUDING ALTERNATIVE MEDICINES

Dr. Subha Ganguly (Editor-in-Chief)

AICRP on Post Harvest Technology (ICAR), Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, 5, Budherhat Road, P.O. Panchasayar, Chakgaria, Kolkata - 700 094, WB, India

SCIENCE AND EDUCATION DEVELOPMENT INSTITUTE, NIGERIA

This first edition published 2014 by
SCIENCE AND EDUCATION DEVELOPMENT INSTITUTE, NIGERIA
2 Church Avenue, Oke Eri Quarters
Oba Ile
P.O.Box 214, Akure
Ondo State
Nigeria
+2348122469297

ISBN: 978 – 978 – 52231 – 8 - 7

SCIENCE AND EDUCATION DEVELOPMENT INSTITUTE, NIGERIA

Vision

To Become a Centre of Excellence Recognized Worldwide in Skill Development and Research

Mission

To Be a Role Model of Academic Excellence in Science and Education

CONTENTS

CHAPTER ONE 1 – 5

HERBAL IMMUNE ENHANCERS AND INDIGENOUS HERBS, PLANTS AND FRUITS AND ITS TRADITIONAL IMPLICATIONS IN THERAPY INCLUDING ALTERNATIVE MEDICINE: AN INTRODUCTION TO READERS

CHAPTER TWO 6 - 20

COMPARTMENT OF PLANT CELL - Md. Rageeb Md. Usman

CHAPTER THREE 21 - 26

DEVELOPMENT OF MOSQUITO REPELLENT FINISHES IN KNITTED FABRICS USING *Rosmarinus officinalis* LEAVES - Banupriya.J and Maheshwari.

CHAPTER FOUR 27 – 38

IN VITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC – AQUA EXTRACT OF T*etradenia riparia* LEAVES - Anthoney Swamy T and Ngule Chrispus Mutuku

CHAPTER FIVE 39 – 79

MEDICINAL PROPERTIES OF INDIGENOUS PLANTS USED IN THE PREPARATION OF TRADITIONAL RICE BEVERAGE "*HANDIA*" AMONG THE TRIBALS OF MAYURBHANJ DISTRICTS, ODISHA, INDIA - Sujogya Kumar Panda, Laxmipriya Padhi and Akshaya Kumar Bastia

CHAPTER SIX 80 – 125

USE OF PLANT EXTRACTS IN PLANT DISEASE MANAGEMENT: ROLE AND SIGNIFICANCE OF CHARACTERIZATION OF SECONDARY METABOLITES FROM HIGHER PLANTS IN PHYTO-DISEASE MANAGEMENT - Enyiukwu, D N., Awurum, A. N., Ononju, C. C and Nwaneri, J.

CHAPTER SEVEN 126 – 177

COMPREHENSION ON USE OF MEDICINAL PLANTS TO CURE DIARRHEA AMONG THE TRIBALS OF MAYURBHANJ DISTRICTS, ODISHA, INDIA
- Laxmipriya Padhi and Sujogya Kumar Panda

CHAPTER EIGHT 178 - 201

MAXIMUM LIKELIHOOD ESTIMATES IN RELIABILITY OF TYPHOID FEVER DRUG IN THE RECOVERY OF TYPHOID FEVER PATIENTS AT AKUSE (A CASE STUDY AT THE AKUSE GOVERNMENT HOSPITAL) - Eric Boahen

ACKNOWLEDGMENTS

The President/CEO wishes to thank members of staff of Science and Education Development Institute, Nigeria for their selfless service in making this publication a reality.

PREFACE

The book aims towards providing the basic and fundamental information to the researchers and scientists worldwide on the vast herbal and natural medicinal treasure available to us derived from plants, herbs and fruits obtained from traditional agricultural practices. This book is dedicated to the professionals of Agriculture, Horticulture and Forestry Sciences and has been composed exclusively for providing first-hand knowledge on the related issues for the development of science and education.

SUBHA GANGULY

Editor-in-Chief

CHAPTER ONE

HERBAL IMMUNE ENHANCERS AND INDIGENOUS HERBS, PLANTS AND FRUITS AND ITS TRADITIONAL IMPLICATIONS IN THERAPY INCLUDING ALTERNATIVE MEDICINE: AN INTRODUCTION TO READERS

Subha Ganguly, Editor-in-Chief (FSEDInst)
AICRP on Post Harvest Technology (ICAR), Department of Fish Processing Technology, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, 5, Budherhat Road, P.O. Panchasayar, Chakgaria, Kolkata - 700 094, WB, India

ABSTRACT

A herbal immunomodulator is a substance which stimulates or suppresses the components of immune system including both innate and adaptive immune responses (Agarwal and Singh 1969). The modulation of immune system by various medicinal plant products has become a subject for scientific investigations currently worldwide.

KEY WORDS: *Herbal immunomodulator, Medicinal plants*

INTRODUCTION

Under Indian scenario, poultry industry has become a means for earning livelihood for the economically distressed farmers in India due to its promising results in productivity and National economy. Poultry rearing is currently the fastest growing industry in our National livestock sector which is benefiting us from production and advantages in prices along with provision of proteinacous food.

Mode of action in immunostimulation of different herbal extracts

Many herbal plant preparations are prescribed to strengthen host resistance (Thatte and Dahanukar 1986). Many useful plants fall under this category. They exhibit immunomodulatory activities. One such plant, *Tinospora cordifolia*, commonly called 'Guduchi' has been examined for its immunomodulatory properties. Guduchi means to rejuvenate dead cells. It is widely used in veterinary folk medicine and has also been claimed to be beneficial according to 'Ayurveda' for the cure of jaundice, skin diseases, diabetes, anemia, emaciations and various infections for its anti-spasmodic, anti-inflammatory, anti-arthritic and anti-allergic properties (Chopra *et al.* 1982). It has also been reported that it improves the phagocytic and bactericidal activities in patients suffering from polymorphism in surgical jaundice (Thatte *at al.* 1989). Kolte *et al.* (2007) studied the effect of feeding *T. cordifolia* in broiler birds which were immunosuppressed with cyclophosphamide. They had found a significant rise in antibody titer against ND virus with augmentation of inflammatory reaction to skin contact sensitivity test. Rege

et al. (1989) and Bishavi *et al.* (2002) have proved the hepato-protective effect of *T. cordifolia.* Manjrekar *et al.* (1999) also found that aqueous extract of *T. cordifolia* is capable of increasing leukocyte count in mice.

Also, *Ocimum sanctum*, commonly known as '*tulsi*' is also used in Ayurveda for various ailments including treatment of allergies. The plant has been reported to evince significant anti-stress properties. The beneficial effects of *O. sanctum* could therefore be due to its direct or indirect effect on the immune system. *O. sanctum* has been reported to modulate humoral immune response by releasing mediators for hypersensitivity reactions (Kujur 2001; Krishnamohan *et al.* 1997; Kumar 2003).

Withania somnifera also fall in this category with many other useful plants. They exhibit immunomodulatory activities. *Withania somnifera* (commonly called 'Ashwagandha') root extracts possess anti-estrogenic, adaptogenic, anti-cancer and anabolic activities having beneficial effects in the treatment of arthritis, geriatric problems and stress. The root of *Asparagus racemosus* (commonly called 'Satavar') possess anti-diarrheal, anti-ulcerative, anti-spasmodic, aphrodisiac, galactogogue and other properties and has therefore gained its importance in Ayurveda, Siddha and Unani systems of medicine (Nadkarni, 1954). It has been observed that feeding *W. somnifera* and *A. racemosus* dried root powder significantly stimulates both humoral and cell mediated immune responses in swiss albino mice by Kuttan and Kuttan (1992). *W. somnifera* and *A. racemosus* extracts increase phagocytic activities of macrophages in vitro (Rege and Dahanukar 1993). There have been studies on the immunomodulatory activities of *W. somnifera* and *A. racemosus* in mice with myelo-suppression induced by cyclophosphamide, azathioprim or prednisolone. Extracts of *W. somnifera* and *A. racemosus* have also shown immunopotentiating effects in cyclophasphamide treated mouse with ascitic sarcoma (Diwanay *et al.* 2004). Kalita and Dutta (1999) reported that maternal antibody was persistently found in sera samples tested against ND virus during the first week of age in broilers. This was attributed to transfer of natural passive immunity in young chicks as demonstrated by Hellar (1975). Muruganandan *et al.* (2001) reported the effects of ethanolic extracts of *W. somnifera* and *A. racemosus* on humoral immune system which was assessed by humoral immune response and cell mediated immune response in mice.

CONCLUSION

The use of various plant extracts and herbal fed additives in a specific dose during the scheduled vaccination regimen may be helpful in obtaining higher protective antibody against different infections including production and development of more effective cell mediate immune response for protection against various bacterial, viral and other diseases. Herbal formulation may be therefore recommended for use as positive immunomodulator in normal and immunocompromized susceptible animals and birds.

REFERENCES

Agarwal S S, Singh V K. 1969. Immnomodulators: a review of studies on Indian Medicinal Plants and Synthetic Peptides. *PINSA*. 65 (3-4): 179-204.

Bishavi B, Roychowdhury S, Ghosh S and Sengupta M. 2002. Hepatoprotective and immunomodulatory properties of *Tinospora cordifolia* in CCl_4 intoxicated mature albino rats *J. Toxicol. Sci*. 27(3): 139-146.

Chopra R N, Chopra L C, Handa K D and Kapur L D. 1982. *Indigenous Drugs of India.* 2nd edn. Dhur & Sons Pvt Ltd, Calcutta, India.

Diwanay S, Chitre D and Patwardhan 2004. Immunoprotection by botanical drugs in cancer chemotherapy. *J. Ethnopharmacol.* 90(1): 49-55.

Hellar E.D. 1975. *Res. Vet. Sci.*18: 117 (Cited by Rao *et al.* 1987). Resistance of maternal antibodies against Newcastle disease virus in chicks from immune parents and its effect on vaccination. *Indian J. Comp. Microbiol. Immunol. Inf. Dis.* 8(3): 106-110.

Kalita D N and Dutta G N. 1999. Immunomodulatory effect of levamisole upon Newcastle disease, pigeon pox and Mark's disease vaccination in broiler chicks. *Indian Vet. J.* 76: 490-492.

Karnataka B C, Shukla S K, Kumar M and Dixit V P. 1993. Immunomodulatory effect of levamisole on the antibody response to RD vaccination. *Indian J. Vet. Med.* 13(2): 48-51.

Kolte A Y, Siddiqui M F and Mode S G. 2007. Immunomodulating effect of *Withania somnifera* and *Tinospora cordifolia* in broiler birds.

Krishnamohan A V, Reddy D B, Sarma B and John Kirubharan J. 1997. Studies on the effects of levamisole against Newcastle disease virus in chicken. *Indian J. Comp. Microbiol. Immunol. Infict. Dis.* 8: 1-6.

Kujur R T. 2001. Evaluation of certain immunomodulatory agents in countering immunosuppressive effects of vaccine strain of infectious bursal disease virus in chicks. *M.V.Sc. thesis.* Rajendra Agricultural Univ., Bihar, India.

Kumar P. 2003. Studies on comparative immunomodulatory effect of herbal preparation and Vitamin E-Se in comparison to Levamisole in broiler chicks. *M.V.Sc. thesis*. Birsa Agricultural Univ., Ranchi, India.

Kuttan G and Kuttan R. 1992. Immunomodulatory activity of a peptide isolated from *Viscum album* extract. *Immunol. Invest.* 21: 285-296.

Manjrekar P N, Jolly C I and Narayan S. 1999. Comparative studies of the immunomodulatory acivities of *Tinospora cordifolia* and *Tinospora sinensis*. *Fitoterapia.* 71: 254-257.

Muruganandan S, Garg H, Lal J, Chandra S and Kumar D. 2001. Studies on the immunostimulant and anti-hepatotoxic activities of Asparagus recemosus root extract. *J. Med. Arom. Pl. Sci.* 22: 49-52.

Nadkarni A.K. 1954. *Indian Materia Medica*, Bombay, Popular book Depot, 3rd edn., 1: 153-155.

Panda S K and Rao A T. 1994. Effect of levamisole on chicken infected with infectious bursal disease (IBD) virus. *Indian Vet. J.* 71: 427-439.

Rege N.N and Dahanukar S.A. 1993. Quantitation of microbicidal activity of mononuclear phagocytes : an in vitro technique. *J. Postgrad. Med.* 39(1): 22-25.

Rege N N, Nazareth H M, Bapat R D and Dhanukar S A. 1989. Modulation of immunosuppression in obstructive jaundice by *Tinospora cordifolia*. *Ind. J. Med. Res.* 90: 478-483.

Renoux and Renoux. 1971. Mechanism of action of some immunomodulatory drugs used in Veterinary Medicine. *Adv. Vet. Sci. Comp. Med.* 35: 43-49. 293

Soppi E, Lassila O, Vilijanen M K, Lehtonon O P and Eskole J. 1979. *Clin. Exp. Immunol.* 38: 609. (Cited by Chakraborty D and Chatterjee A. 1998. Studies on immunomodulatory effect of Levamisole in Newcastle disease vaccinated chicks. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 19: 85-87).

Thatte U M and Dahanukar S A. 1986. Ayurveda and contemporary scientific thought. *Trends in Pharmacol. Sci.* 17: 248-257.

Thatte U M and Dahanukar S A. 1989. Immunotherapeutic modification of experimental infection by Indian medicinal plants. *Phytothe. Res.* 3: 43-49.

Vyas G P, Dholakia P M and Kathiria L G. 1987. Studies on immunomodulation by levamisole along with vaccination in chicks against Ranikhet disease. *Indian Vet. J.* 64: 456-462.

Suggested Readings:

Ganguly, S. and Prasad A. (2010) Role of plant extracts and cow urine distillate as immunomodulator in comparison to levamisole – a Review. *J. Immunol. Immunopathol.*, 12(2): 91-94.

Prasad, Arun and Ganguly, Subha (2012) Herbal Immunomodulators. AV Akademikerverlag GmbH & Co. KG, Saarbrücken, Germany *with trademark* LAP LAMBERT Academic Publishing. ISBN 978-3-659-30401-9.

Ganguly, Subha (2013) A Handbook on Traditional Medicinal Plants, Herbs and Fruits in Indian Agriculture and Forestry. 1st Edition. *International E-Publication*. ISBN 978-81-927544-5-1. Official E-Book (*Section:* Agriculture & Forestry Sciences). Publication of the *International Science Congress Association*, Indore, UP, India.

CHAPTER TWO

COMPARTMENT OF PLANT CELL

Md. Rageeb Md. Usman
Department of Pharmacognosy, Smt. S. S. Patil College of Pharmacy, Chopda, Maharashtra, 2/899 SURMAZ Villa, KGN Colony, Chopda, Maharashtra, India.

A. PLANT TISSUE

Types of plant tissue

Meristematic tissue

Apical meristems
Lateral meristems
Intercalary meristems

Permanent tissue

Simple permanent tissue
Parenchyma
Collenchyma
Sclerenchyma

Complex permanent tissue

Xylem
Phloem

Protective tissue

Epidermis
Cork (phellem)

Meristematic tissue

Apical meristems

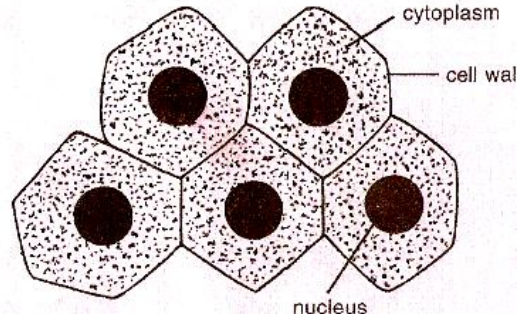
These are situated at the growing tip of the stems & roots, i.e., at shoot apex & root apex. Apical meristems are also found at apices of the leaves. It brings about the elongation of the root & stem. It results in increase in the height of the plant, which is called primary growth.

Lateral meristems

These are found beneath the bark (cork cambium) & in vascular bundles of dicot roots & stems (cambium).They occurs in thin layers. Cambium is the region which is responsible for growth in thickness. It causes the organ (stem or root) to increase in diameter & girth. This is called secondary growth.

Intercalary meristems
They are located at the base of leaves or internodes, e.g., Stem of grasses & other monocots.

It produces an increase of length of organ.



Meristematic Tissue

Nature-cells of meristems divide continuously & help in increasing the length girth of the plant. These cells show the following characteristics:

1. The cells of meristematic tissue are similar in structure & have thin cellulose cell walls.
2. The meristematic cells may be spherical, oval, polygonal or rectangular in shape.
3. The meristematic cells are compactly arranged & do not contain any intercellular space between them.
4. Each meristematic cell contains dense or abundant cytoplasm & a single large nucleus.
5. The meristematic cells contain few vacuoles or no vacuoles at all.
6. Occurrence-Meristematic tissues are growth tissues & are found in those regions of the plant that grow. According to their position in the plant, meristems are apical, lateral & intercalary.
7. Function-the main function of meristematic tissue is to continuously form a number of new cells.

Permanent tissue
These tissues derived from the meristematic tissues but their cells have lost the power of division & have attained their definite forms. Permanent tissues are classified into two-simple & complex.

Simple permanent tissue
These tissues are composed of cells which are structurally & functionally similar. Thus, these tissues are all made of one type of cells.

They are of three types
I. Parenchyma
II. Collenchyma
III. Sclerenchyma

Complex permanent tissue

The complex tissues consist of more than one type of cells. All this co-ordinate to perform a common function. Complex tissues transport water, mineral salts (nutrients) & food material to various parts of plant body. Complex tissues are of following two types:
I. Xylem or wood
II. Phloem or bast
Xylem & phloem are both conducting tissues & also known as vascular tissues; together both of them constitute vascular bundles.

Simple permanent tissue

1. Parenchyma

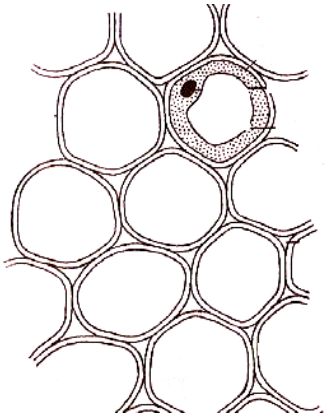
Living & possess the power of their division. The cells are rounded or isodiametric, i.e., equally expanded on all sides. The parenchymatous cells are oval, round, polygonal or elongated in shape. The cell wall is thin & encloses a dense cytoplasm which contains a small nucleus & surrounds a large central vacuole. In other words, parenchyma cells have living protoplasm. Intercellular spaces are abundant

Occurrence

The parenchyma is widely distributed in plant body such as stem, roots. Leaves, flowers & fruits. Thus, the parenchyma tissue is found in the soft parts of the plant such as cortex of roots, ground tissues in stems & mesophyll of leaves. It is also distributed in pith, medullary rays & packing tissue in xylem & phloem.

Functions

1. Parenchyma serves as a packing tissue to fill the spaces between other tissues & maintain the shape & firmness of the plant due to its turgid cells.
2. Due to turgidity property, parenchyma forms the main means of support to the stem of herbaceous plants.
3. The main function of parenchyma is to store & assimilate food. Parenchyma serves as food storage tissue.
4. Transport of materials occurs through cells or cell walls of parenchyma cells.
5. Parenchyma cells are metabolically active their intercellular air spaces allow gaseous exchange.



Parenchyma

2. Collenchyma

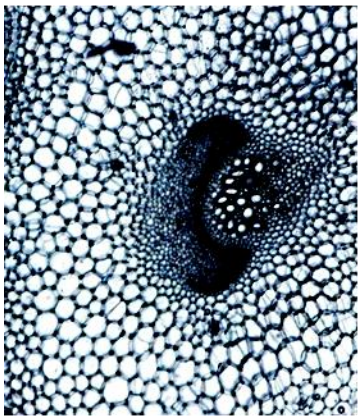
Nature-collenchyma tissue consists of living cells. It shows many of the features of parenchyma but is characterised by the deposition of extra cellulose at the corners of the cells. In collenchyma, intercellular spaces are generally absent. Collenchymas cells are elongated in shape. They often contain a few chloroplasts.

Occurrence

The cells of collenchyma are located below the epidermis of dicotyledons stem & petiole. These cells also occur in midribs of dicot leaves. Collenchyma is absent in monocot stems, roots & leaves.

Functions

Collenchyma is a mechanical tissue; it provides mechanical support & elasticity. Thus, collenchyma provides tensile strength with flexibility to those organs in which it is found. It allows easy bending in various parts of a plant without actually breaking it. When cells of collenchyma contain some chloroplasts, they manufacture sugar & starch.



Collenchyma

Sclerenchyma

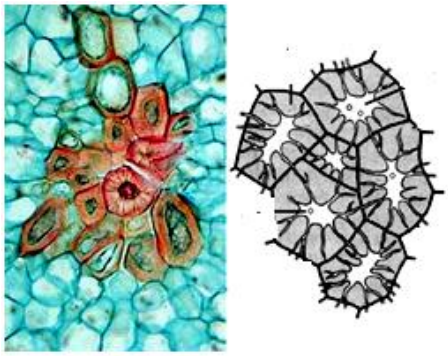
Nature-sclerenchyma cells are dead cells & they are devoid of protoplasm. The walls of cells of sclerenchyma are greatly thickened with deposition of lignin. Such cells are called lignified. Due to excessive thickening of the wall of a sclerenchyma cells, its cell cavity or lumen becomes nearly absent. The cells of sclerenchyma are closely packed without intercellular spaces.

Occurrence

The sclerenchyma occurs in abundance either in patches or definite layers. They are found in stems, roots, veins of leaves, hard covering of seeds & nuts. Sclereids form the gritty part of the most of the ripe fruits & contribute hardness to the seed coat & nutshells.

Functions

The sclerenchyma is mainly mechanical & protective in function. It gives strength, rigidity, exibility & elasticity to the plant body &, thus, enables it to withstand various strains.



Sclerenchyma

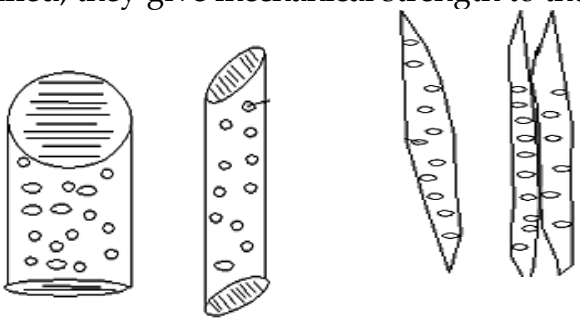
Complex permanent tissue

Xylem

Nature-xylem is a vascular & mechanical tissue. In other words, it is a conducting tissue. Xylem is composed of cells of four different types like, tracheids; vessels ortracheae, xylem parenchyma; xylem sclerenchyma. Except xylem parenchyma, all other xylem elements are dead & bounded by thick lignified walls. Of these four types of cells of xylem are most important cells are vessels. Vessels are very long tube-like structures formed by a row of cells placed end to end. Tracheids are elongated cells with tapering ends. They conduct water.

Functions

The main function of xylem is to carry water & minerals salts upward from the root to different parts of shoots. Since walls of tracheids, vessels & sclerenchyma of xylem are lignified, they give mechanical strength to the plant body.



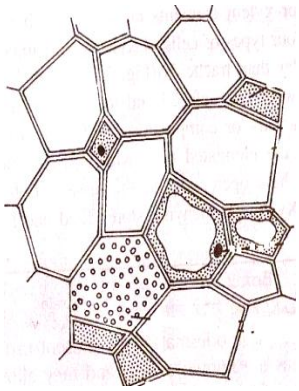
Xylem

Phloem

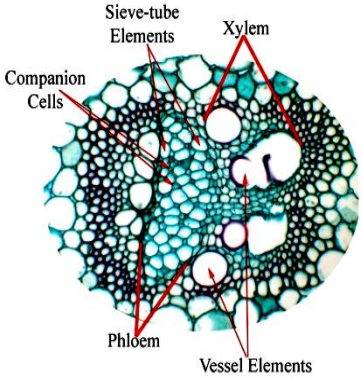
Nature-like xylem, it contains tubes but has no mechanical function. Phloem is composed of following four elements or cells like, sieve tubes, companion cells, and phloem parenchyma& phloem fibres. Except for phloem fibres, phloem cells are living cells.

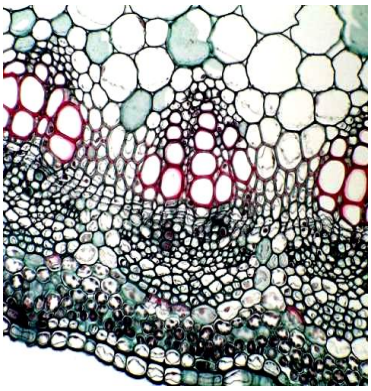
Functions

Phloem transport photosynthetically prepared food materials from the leaves to the storage organs & later from storage organs to the growing regions of the plant body.



Phloem





Vascular Bundles with Xylem & Phloem

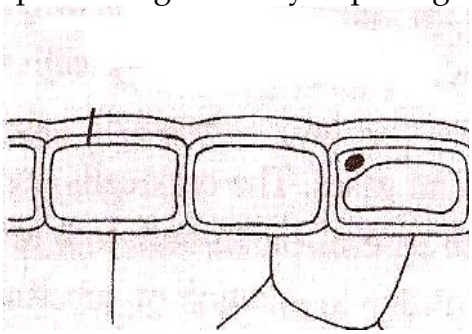
Protective tissue

Epidermis

It is usually present in the outermost layer of the plant body such as leaves, flowers, stem & roots. Epidermis is one cell thick & is covered with cuticle. Cuticle is a water proof layer of a waxy substance called cutin which is secreted by epidermal cells. Cuticles possess variable thickness in plants such as it is thicker in xerophytic plants.

Cells of epidermis are elongated & flattened & do not contain any intercellular space between them. Their inner contents are similar to parenchyma cells.

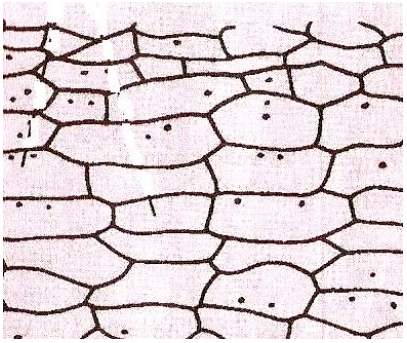
The main function of epidermis is to protect the plant from desiccation & infection. In fact, cuticle of epidermis helps to reduce water loss by evaporation from the plant surface as well as helping in preventing the entry of pathogens



Epidermis

Cork

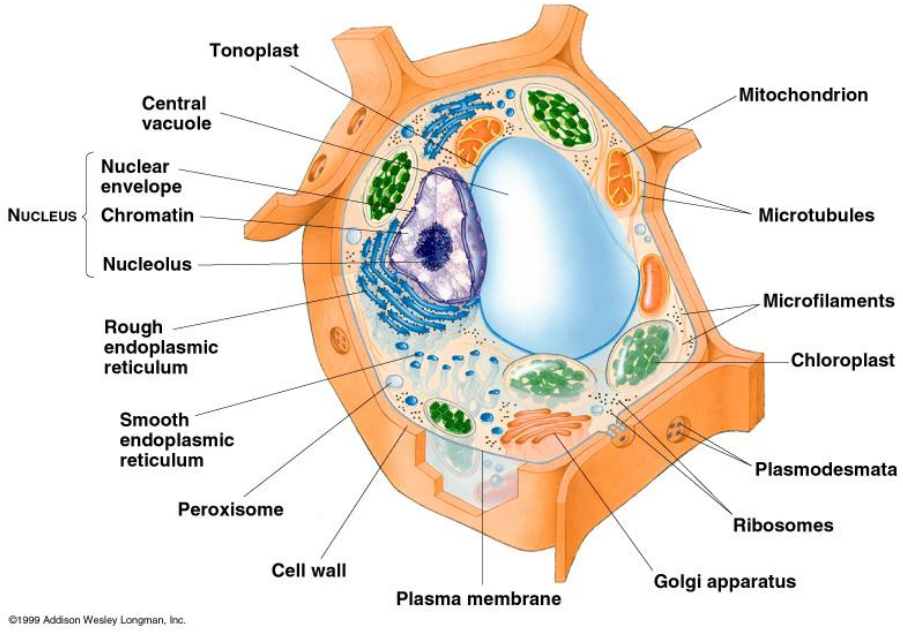
As plants grow older, the outer protective tissue undergoes certain changes. A strip of secondary meristems, called phellogen or cork cambium replaces epidermis of stem. Cork cambium is a simple tissue having only one type of cells. The cells of cork cambium are rectangular & their protoplasts are vacuolated & contain tannins & chloroplasts. Cork cambium gives off new cells on its both sides, thus, forming cork on the outer side & the secondary cortex or Phelloderm on the inner side. The layer of cells which is cut by cork cambium on the outer side ultimately becomes several layered thick cork or the bark of trees. Cells of cork are dead & compactly arranged without intercellular spaces. The walls of cork cells are heavily thickened by deposition of an organic substance, called suberin. Suberin makes these cells impermeable to water & gases. The cork cells do not contain protoplasm but are filled with resin or tannins. In case of onion bulb too, in the skin of onion the cell walls become thick & water proof due to addition of suberin. Cork is protective in function. Cork cells prevent desiccation, infection & mechanical



Cork

B. THE CELL

The elementary organs from which the body of the plant is constructed are the cells. Most plants (all the more highly organized ones) consist of numerous cells. Among the lower plants there are, however, many which arc formed of but single cells, some of which assume the most manifold forms, branch abundantly, and, indeed, without being in any manner divided by lateral walls, imitate a stem, leaf, and root.



Contains of the Cell

Cell-contents with the distinction already made the cell contents may be grouped into two classes: (1) Protoplasmic, or those in which the life-processes of the plant, or cell, are manifested, and (2) non-protoplasmic, or those which are the direct or indirect products of the protoplast. The first class includes the protoplasm with its various differentiated parts, and the second, the various carbohydrates (starches and sugars), calcium, oxalate, aleurone, tannin, oil, and a number of other substances.

Cell Wall

It is rigid wall made up of cellulose, proteins, and carbohydrates

Function: boundary around the plant cell outside of the cell membrane that provides structure and support

Protoplasm

Protoplasm occurs as a more or less semi fluid, slimy, granular, or foam-like substance, which lies close to the walls of the cell as a relatively thin layer and surrounding a large central cavity or vacuole filled with cell-sap. or it may be distributed in the form of threads or bands forming a kind of network enclosing smaller vacuoles. Protoplasm consists of two comparatively well differentiated portions: (1) Certain more or less distinct bodies which appear to have particular functions and to which a great deal of study has been given, as the nucleus and plastids, and (2) a less dense portion which may be looked upon as the ground substance of the protoplast and which is now commonly referred to as the cytoplasm. These differentiated bodies and the cytoplasm are intimately associated and interdependent. The nucleus and cytoplasm are present in all living cells and it is through their special activities that cell division takes place. When in addition plastids are present, constructive metabolism takes place, whereby complex substances are formed from simpler ones. Besides the nucleus and plastids other protoplasmic structures are sometimes found embedded in the cytoplasm

Centrospheres

Small spherical bodies are associated with the nucleus and appear to be concerned in cell division. There are in fact quite a number of minute bodies in the cytoplasm which may be always present or only under certain conditions, and which are grouped under the general name of microsomes or microsomata.

Chemically protoplasm is an extremely complex substance, but does not appear to have a definite molecular structure of its own, being composed in large measure of proteins, a class of organic compounds which always contain nitrogen, and frequently phosphorus and sulphur. The molecule of the proteins is large and more or less unstable, and hence subject to rapid changes and a variety of combinations, and it is to these interactions that the vital activities of the plant are attributed Nucleus. The nucleus consists of a ground substance in which is embedded a network composed of threads containing a granular material known as chromatin, and generally one or more spherical bodies called nucleoles, the while being enclosed by a delicate membrane. The chromatin threads are readily stained by some of the aniline dyes, and are mainly composed of nucleins (proteins) rich in phosphorus, which by some writers are supposed to be essential constituents of the nucleus and necessary to the life of the protoplast. Chromatin is constant in the nucleus and prior to cell division the threads become organized into bodies of a definite number and shape known as chromosomes.

Plastids

The plastids or chromatoid hores form a group of differentiated protoplasmic bodies found in the cytoplasm (Frontispiece) and are associated with it in the building up of complex organic compounds, as starch, oil and proteins. The term chromatophore

means colour-bearer, but applies also to those plastids which may be colourless at one stage and pigmented at another.

Hence we may speak of colourless chromatophores. According to the position of the cells in which these bodies occur and the functions they perform, they vary in colour three distinct kinds being recognized. (1) In the egg-cell and in the cells of roots, rhizomes and seeds the plastids are colourless and are called leucoplastids. (2) When they occur in cells which are more or less exposed to light and produce the green pigment called chlorophyll, they are known as chloroplastid or chloroplasts. (3) In other cases, independently of the position of the cells as to light or darkness, the plastids develop a yellowish or orange-colored principle, which may be termed chlorophyll, and are known as chromoplastids.

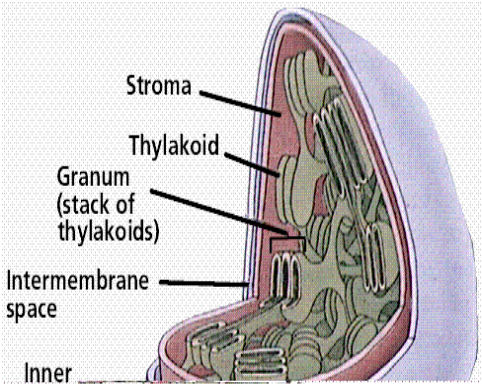
Chromo plastids.

Chloroplastid is found in all plants except Fungi and non-chlorophyllous flowering plants, and chromoplastids in all plants except Fungi. Plastids vary in form from more or less spherical to polygonal or irregular-shaped bodies, and they increase in number by simple fission. They suffer decomposition much more readily than the nucleus, and are found in dried material in a more or less altered condition.

Leucoplastids. The chief function of the leucoplastids is that of building up reserve starches or those stored by the plant for food, and they may be best studied in the common potato tuber, rhizome of iris, and the over ground tubers. The reserve starches are formed by the leucoplastids from sugar and other soluble carbohydrates. The chloroplastid occurs in all the green parts of plants. They vary from 3 to 11 /x in diameter and are more or less spherical or lenticular in shape, except in the Algae, where they are large and in the shape of bands or disks, and generally spoken of as chromatophores. Chloroplastid are found in greater abundance in the cells near the upper surface of the leaf than upon the under surface, the proportion being about five to one. These grains upon close examination are found to consist of (1) a colourless stroma, or liquid, in which are embedded (2) green granules; (3) colourless granules; (4) protein masses; (5) starch grains; and (6) a membrane which surrounds the whole. The green granules are looked upon as the CO_2 assimilation bodies, the colourless grains are supposed to assist in the storing of starch or in the production of diastase, the conditions for these processes being directly opposite, i.e., when CO_2 assimilation is active, starch is stored, and when this process is not going on. As at night, diastase is produced and the starch is dissolved. The protein grains may be in the nature of a reserve material of the plastid and are also probably formed as a result of CO_2 as simulation. While the protoplasm has been termed by Huxley " The physical basis of life," the chloroplastid has been spoken of as the mill which supplies the world with its food, for it is by the process of photosynthesis that the energy of the sun is converted

into vital energy, and starch and other products formed, which become not only the source of food for the plant itself, but also the source of the food-supply of the animals which feed upon plants. It other words, horse-power is derived from the energy of the sun which is stored by the chloroplastid in the plant.

Chromoplastids. In many cases, as in roots, like those of carrot, or flowers and fruits, which are yellowish or orange colored, there is present a corresponding yellow pigment, and to this class of pigments the name chlorophyll may be applied. Some of these pigments, as the carotin in carrot, have lien isolated in a crystalline condition Chromoplastids usually contain, as first pointed out by Schimper and Meyer, protein substances in the form of crystal-like bodies; starch-grains may also be present. The chromo plastids are variable in shape and in other ways are markedly different from the chloroplastid. They are more unstable than the chloroplastid, and are formed in underground parts of the plant, as in roots, as well as in parts exposed to the light, as in the flower. Their formation frequently follows that of the chloroplastid, as in the ripening of certain yellow fruits, such as apples, oranges, persimmons, etc.



Chromo Plastids

Plastid pigments

They are distinguished from all other colour substances in the plant by the fact that they are insoluble in water and soluble in ether, chloroform and similar solvents. In fact they are but little affected by the usual chemical reagents under ordinary conditions. Apart from the difference in colour, the yellow pigment (chlorophyll) is distinguished from the green (chlorophyll) by the fact that the latter is said to contain nitrogen, and also by their difference in behaviour when examined spectroscopically, chlorophyll giving several distinct bands in the yellow and orange portion of the spectrum, which arc wanting in the spectrum of the yellow principle.

Non-protoplasmic cell-contents

The non-protoplasmic constituents of plants may be said to differ from the protoplasmic cell-contents in two important particulars, namely, structure and function.

For convenience in considering them here, they may be grouped as follows : (1) Those of definite form including (a) those which are colloidal or crystalloid, as starch and inulin; (b) those which are crystalline, as the sugars, alkaloids, glucosides, calcium oxalate (c) composite bodies, as aleurone grains, which are made up of a number of different substances. (2) Those of more or less indefinite form, including tannin, gums and mucilages, fixed and volatile oils, resins, gum-resins, oleo-resins, balsams, and also silica and calcium carbonate.

Substances definite in form. Colloidal or crystalloid.
Starch is the first visible product of photosynthesis although it is probable that simpler intermediate products are first formed. This substance is formed in the chloroplastid and is known as assimilation starch. Starch grains are usually found in the interior of the chloroplastid, but may attain such a size that they burst through the boundary wall of the plastid, which latter in the final stage of the growth of the starch grain forms a crescent-shaped disk attached to one end of grain, as in Pellionia. Starch is changed into soluble carbohydrates by the aid of ferments and probably other substances, and in this form is transported to those portions of the plant requiring food. The starch in the medullary rays and in other cells of the wood and bark of plants is distinguished by being in the form of rather small and nearly spherical grains. In rhizomes, tubers, bulbs and seeds the grains are, as a rule, quite large, and possess more or less distinct characteristics for the plant in which they are found.

Crystalline substances.
The sugars constitute a group of crystalline principles of wide distribution. They occur in the cell-sap, from which by evaporation or on treatment with alcohol they may be crystallized out. Quite a large number of distinct principles belonging to this class have been recognized, of which the following may be mentioned

Dextrose
(grape-sugar or dextro-glucose) is found in sweet fruits, the nectarines of the flowers, and stems and leaves of various plants. It crystallizes in needles and varies in amount from i to 2 per cent, (in peaches), to 30 per cent

Sucrose
(saccharine or cane-sugar) is found rather widely distributed, as in the stems of corn, sorghum and the sugar-cane ; in roots, as the sugar-beet ; in the sap of certain trees, as sugarmaple and some of the palms

Maltose
It is found in the germinating grains of cereals (see malt); it forms colourless, needle-shaped crystals resembling those of dextrose, and forms compounds with calcium, strontium, barium and acetic acid.

Cell-sap

The majority of the other colour-substances found in the higher plants besides the green and yellow principles previously mentioned occur in solution in the cell-sap, and may be in the nature of secondary substances derived from the plastid pigments, or they may be produced directly by the protoplasm. Upon making sections of the tissues containing cell sap colour substances, not infrequently strikingly contrasting colours are observed in contiguous cells; as in the petals of the poppy and petals of certain lilies, where we find some cells of a deep purple colour, others of a deep red and still others of intermediate shades. Calcium oxalate is found in many of the higher plants, and in the algs and fungi as well; while in the mosses, ferns, grasses and sedges it is seldom found. It occurs in plants in crystals of either the monoclinic or tetragonal system. The crystals dissolve in any of the mineral acids without effervescence and their identity is usually confirmed by the use of dilute hydrochloric acid. The crystals of the monoclinic system are rather widely distributed, while those of the tetragonal system are less frequent in their occurrence, being found in species of Allium, crystals belonging to the monoclinic system include a number of forms, as follows: (1) Rosette aggregates, or what are commonly termed rosette-shaped crystals; (2) prisms, pyramids and elongated or irregular polygonal-shaped crystals (3) crystal-fibers (4) raphides; (5) sphenoid micro-crystals.

Rosette aggregates of calcium oxalate

It consists of numerous small prisms and pyramids, or hemihedral crystals more or less regularly arranged around a central axis, have the appearance of a rosette or star. The development of these aggregates may is readily observed in the stem of Datura. Crystals of this class are more widely distributed than any of the others, and are found in a number of drugs. Monoclinic prisms and pyramids are also widely distributed and are frequently so in form that they are of an elongated or irregular polygonal shape. The crystals of this group are sometimes mistaken for silica, owing to the fact that in some instances the lumen of the cell is completely filled by the crystal, and the inner wall having the contour of the crystal, it is impossible to determine whether the crystal is affected by the use of hydrochloric acid. It should be stated in this connection that silica never occurs as a cell-content in sharp, angular crystals, but either in more or less ellipsoidal or irregular hollow masses, or in somewhat solid, irregularly branching masses.

Crystal Fibers.

In quite a number of drugs a single monoclinic prism occurs in each of the parenchyma cells adjoining the sclerenchymatous fibers, and to this single longitudinal row of superimposed cells the name crystal fiber has been applied

Raphides

Raphides are groups of needle-shaped crystals which are found in various plants .These have been mistaken by several observers for calcium phosphate. Calcium phosphate, however, occurs in plants either in solution or in combination with protein substance. The cells containing raphides are long, thin-walled and contain sooner or later a mucilage, which arises from the cell-sap and behaves with reagents much like cherry gum.

Amorphous substances

Cystoliths.

Occasionally cells are found among the parenchyma or in the inner row of the epidermal cells on the upper side of the leaf, the walls of which form an inward protrusion in cell and impregnated with and encrusted by calcium Carl ovate, giving rise to more or less stalked bodies known as Cystoliths

The calcium carbonate

Calcium carbonate dissolves on the application of acetic acid, leaving a core which responds to the tests for cellulose. Cystoliths are not of common occurrence, being found with but few exceptions in the two families Acanthaceae and Moraceae, and in a few species of the Cucurbitacese. In the leaves of the cultivated ruber plant the Cystoliths have long stalks, whereas in cannabis indica, they are sessile.

Tannin and Tannoids

Tannins are astringent principles which belong to the class of phenol acids and give blue or green precipitates with iron salts. The Tannoids, in addition, precipitate aluminous compounds, and when applied to animal hides convert them into leather. These principles are widely distributed, occurring dissolved in the cell-sap, in parenchyma cells or in distinct reservoirs or vessels, and vary in amount from T per cent, or less to as high as 70 per cent, in Chinese galls. Tannin may be precipitated in the plant cells by copper acetate.

Mucilages and Gums

By the terms mucilages and gums are meant those substances which are soluble in water, or swell very perceptibly in it, and which, upon the addition of alcohol, are precipitated in the form of a more or less amorphous or granular mass.

REFERENCES

Botanic drugs Their materia medica, pharmacology And therapeuticspublished by The therapeutic digest publishing co. Cincinnati, ohio 1917.

Herbal Pharmacognosy 312,Practical manual Coordinators: Miss M. Hess and Miss S. Nyati.

Medicinal plants, kerala agricultural university Aromatic and medicinal plants research station, 1998.

The Ayurvedic pharmacopoeia of India part - ii (formulations) Volume – I. First edition Government.

The Principles of Pharmacognosy An introduction to the study of the crude substances of the vegetable kingdom, New York William Wood & Company 1887.

The Ayurvedic Pharmacopoeia of India Part- I, Volume – i, ii, iii, iv, government of India Ministry of Health and Family Welfare Department of Ayush.

CHAPTER THREE

DEVELOPMENT OF MOSQUITO REPELLENT FINISHES IN KNITTED FABRICS USING *Rosmarinus officinalis* LEAVES

Banupriya.J and Maheshwari.V
Costume Design and Fashion, PSG College of Arts and Science/ Bharathiar University, India.

ABSTRACT

The textile materials were considered primarily for economic and functional point of view some end users in particular demands on the safety of textiles for the health. An insect-repellent helped to prevent and control the outbreak of insect-home disease such as malaria, dengue fever. The most herbal plants contain compounds that are preventing attack from phytophagous regulators. Insect repellent textiles are also a part of protective textiles which help in protection from the species that are prone to cause damage in some or the other manner. These textile products find their application over a wide range. The knitted fabrics were finished with mosquito repellent test by excite chamber. The treated fabrics show 100% mosquito repellent efficiency. The finished fabrics were tested for laundering process they retained their activity until 16 washes. So these types of plant based mosquito repellents has been used for generations in practice as a personal protection against mosquito.

***KEYWORDS**: Eco-friendly fabrics, herbal plants, laundering properties.*

INTRODUCTION

Protective textiles are among one such smart application of smart technology in textiles. Protective textiles refer to those textile products which have a functionality of giving protection from something in some or the other sense. Mosquitoes have a complex method of detecting hosts and different types of mosquitoes react to different stimuli. At present, there are very few durable repellents that can be applied to clothing and almost all the repellents are designed to be applied directly on the skin. This poses a great risk to the individuals using them and hence, with a view to reducing this risk and at the same time meeting the needs of industry. Basically mosquito repelling textiles are the ones which have a character of repelling mosquitoes. This feature was developed as a need in sense of protection from the mosquitoes in the areas which are habitats of the mosquitoes and are prone to disease like malaria. Global warming has resulted in the spread of mosquitoes from tropical regions to rest of the world resulting in spread of viral infection to different parts of the world. Anti- mosquito finishing on textile products can suppress mosquito-transmitted diseases such as West Nile fever; malaria.

Herbs are available in a variety of forms, including fresh, dried, in tablets or capsules, or bottled in liquid form. Buy them individually or in mixtures formulated for specific conditions. Finishing is the general term for a multitude of processes and treatments which a fabric may undergo after it has been knitted. Finishes are also categorized by their degree of performance.

Cotton is the natural vegetable fiber of great economic importance as a raw material for cloth. Bamboo is grown using methods and materials that have low impact on the environment.

MATERIALS AND METHODS

Fabric selection

The fabric used as the textile substrates in the present research work was 50's combed bamboo/cotton blended knitted fabrics.

Pretreatment

The material is treated with soap at 50 ^{0}C for 20 minutes to remove the dirt on the untreated fabric with water. The soap solution is added into water in the proportion of 3: 1.Then the material is given hot wash and cold wash. The M: L is 1:30.

Selection of the Medicinal Valuable Herbs

The herbal plant were identified and collected from the natural resources in a pure form. The following plant was chosen for the study *Rosemary*. The procedure begins with the selection of natural herb, which was screened and identified. The extract was tested for its Mosquito Repellent which was done by Excito chamber method.

Rosemary has been around for a long time, and therefore has a long list of claims regarding its medicinal uses, including use as a tonic, a digestive aid, to treat depression, headache, and muscle spam, and as an expectorant, promoter of menstrual flow, and stimulant for production of bile.

Extraction of Rosemary leaves

Methanol Extraction

For extraction, 6g of dry powder from each herb (*Rosemary*) was taken and mixed into 50ml of 80% Methanol. The container was closed and kept for over night. After over night incubation, the extract was filtered through filter paper and evaporated to concentrate the extract. The extract was finished on the fabric by dip dry method and tested for its mosquito repellency activity.

Collection of mosquitoes

Anopheles mosquitoes were identified based on morphologic keys and they were collected during the evening time. All the mosquitoes were starved of blood and sugar for 4 hours.

Mosquito Repellency Behavioral Test

Specially designed two excito repellency test chambers were used to evaluate the efficiency of repellency activity. The wooden outer chamber of excito-repellency testing device measures 34 cm × 32 cm × 32 cm and faces the front panel with the single escape portal. The box is composed of a rear door cover, an inner Plexiglas glass panel with a rubber latex-sealed door, a Plexiglas holding frame, a screened inner chamber, an outer chamber, a front door, and an exit portal slot. Mosquitoes were deprived of all nutrition and water for a minimum of 4 hours before exposure. Laboratory tests were performed during daylight hours only and each test was replicated four times. Observations were taken at one-minute interval for 30 minutes. After each test was completed, the number of Escaped specimens and those remaining inside the chamber was recorded separately for each exposure chamber, external holding cage, and paired control chamber. Escaped specimens and those remaining inside the chamber, for the treated samples, were held separately in small holding containers with food and water. The repellency were calculated by below formula:

$$\text{Efficiency of Mosquito repellency (\%)} = \frac{\text{No. of Specimen escaped + No. of specimen dead}}{\text{No. of Specimen exposed}} \text{ X 100}$$

Fig-1 shows the Excito chamber

Wash durability test

The *rosemary* extract finished samples was then subjected for 16 machine washes and the washed fabric was tested for its mosquito repellency efficiency using excite chamber method.

RESULTS AND DISCUSSION

The results shows that the mosquito repellent activity was the highest with Rosemary extract finished samples. The mosquito repellent activity for the fabrics was determined by examining the mortality rates of the mosquitoes after exposure to the leaf extracts.

Percentage of mosquito repellency

Table-1 shows the percentage of mosquito repellency.

S.No.	methods	samples	Mosquito Repellency in %
1	Untreated	Controlled	0
2	herbal	*Rosemary*	88

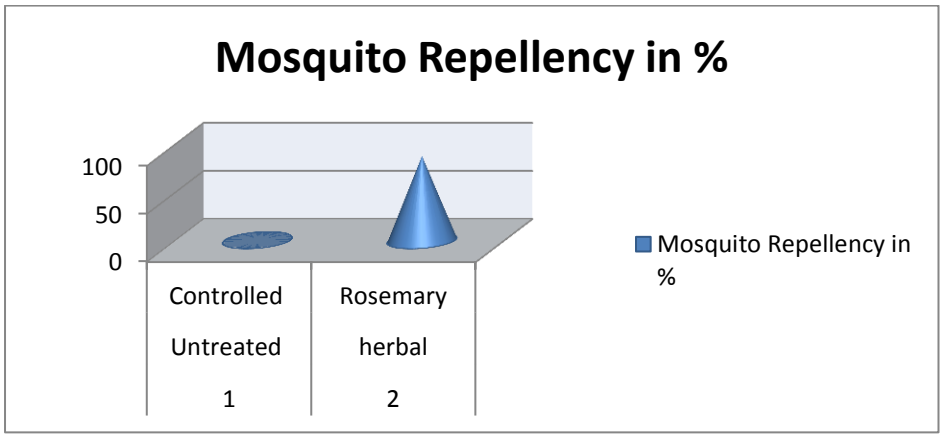


Fig-2 represents the herbal extracts treated fabrics percentage.

Wash durability

The laundering durability of the treated fabrics were tested with excite chamber after each and every washes it was calculated using formula. The coated fabrics were with stand nearly up to 16 washes.

Table-2 shows the wash durability test for treated fabrics.

S.No	Samples	Mosquito Repellency % Herbal
1.	Untreated	0
2.	Before laundering	88
3.	After launderings 4	88
4.	After launderings 8	68
5.	After launderings 12	60
6.	After launderings 16	48
7.	After launderings 20	20

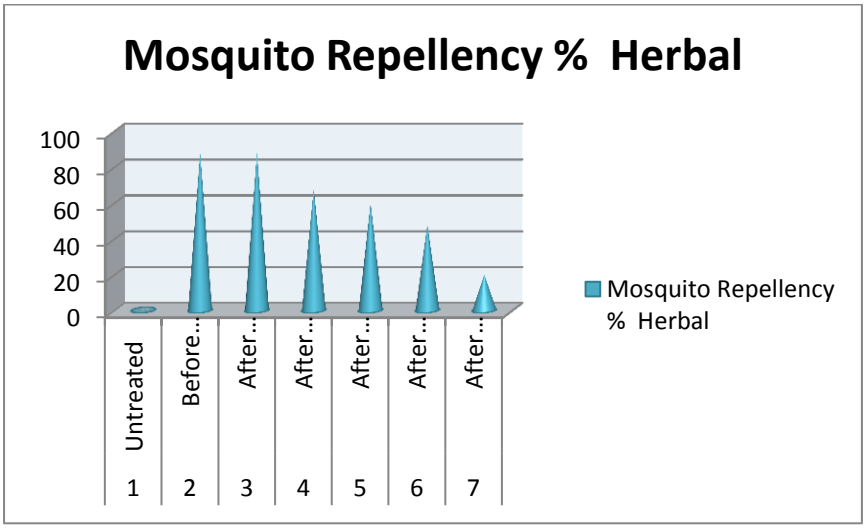


Fig-3 represents the percentage of washing property.

CONCLUSION

From the study it was concluded that *rosemary* herbal extracts treated fabrics eco-friendly, bio-degradable and non-toxic to the skin. Apart from the industrial use, mosquito repellent finish on textiles has become essential in our day today life to live in

free diseases and hygienic atmosphere. The finish has excellent potential in various textile uses baby care products and Night wears etc.

REFERENCE

Butter Worth, (1964), Review of Textile Process, Butter Worth And Co. Publishers Ltd, Bangalore, P-330

Carey AF, Wang G, Su CY, Zwiebel LJ, Carlson JR. Odorant reception in the malaria mosquito *Anopheles gambiae* Nature. 2010; 464:66–71.

Elementary idea of textile dyeing, printing and finishing, kanwar varinder pal singh, Kalyani Publishers, 2004, pg.no-5, 29

Hallem EA, Dahanukar A, Carlson JR., (2006). "Insect odour and taste receptors" *Annu Rev Entomol*. Vol.51. P.113–135

Shell ER. Resurgence of a deadly disease. Atlantic Monthly. August 1997:45-60. Malaria. Fact sheet. No. 94. Geneva: World Health Organization, 1999. (Accessed May 3, 2002,

The Journal of Textile Association.

Taubes G. A mosquito bites back. New York Times Magazine. August 24, 997:40-6.

CHAPTER FOUR

IN VITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC – AQUA EXTRACT OF T*etradenia riparia* LEAVES

Anthoney Swamy T and Ngule Chrispus Mutuku
Department of Chemistry, University of Eastern Africa Baraton, P.O. Box 2500, Eldoret -30100, Kenya.

ABSTRACT

Ethnobotanical medicine has attracted great interest due to the belief that it is safe, cheap and more dependable than aliphatic drugs, which have adverse side effects. The current study was conducted to analyze the antibacterial activity of Tetradenia riparia leaves hydroalcoholic extract. The plant sample was extracted using methanol and water in the ratio of 9:1. From the study the plant Tetradenia riparia was found to inhibit the growth of Escherichia coli with a zone of inhibition of 11.67 ± 0.882, Serratia liquefaciens zone of inhibition of 12.67 ± 0.667, Bacillus cereus 28.00 ± 1.154, Enterobacter aerogenes 8.67±0.882 and Proteus vulgaris 11.00 ± 0.577. The data collected and documented in this paper is a scientific justification that the plant Tetradenia riparia can be used to treat against various diseases caused by Escherichia coli, Serratia liquefaciens, Bacillus cereus, Enterobacter aerogenes and Proteus vulgaris. However, further research is needed to isolate the active compounds identify their structure, their mode of action to the microorganisms and their effect in the in vivo environment.

KEYWORDS: *Tetradenia riparia, Antibacterial, Medicinal herbs, Leaves.*

INTRODUCTION

Nature is a paradise of medicinal solutions to all ailments affecting human beings through medicinal plants. Medicinal plants are being used widely to treat against the currently widespread strains of drug resistant bacteria. Scientists all over the world are working hard to provide scientific justification on the traditional use of medicinal plants to treat against the ailments affecting human beings

Pharmacological studies have reported appealing results showing the importance of using plant extract to treat diseases. The reports have shown that plants can be used as antitumor, anti-inflammatory, antibacterial activity, anti-hyperlipidemic, anti-hyperglycemic and hypoglycemic activity (Kaoli and Kauli, 2011). The antibacterial activity of plants has been associated with the presence of certain compounds referred

to as phytochemicals such as tannins, saponins, terpenoids, flavonoids, phenols and alkaloids (Ngule et al., 2013; Nyaberi et al., 2008).

All chemicals found in plants are potential drugs. Certain tree barks produce chemicals that discourage caterpillars from feeding on them, a good example being the Indian neem tree, which keeps off desert locusts. The twigs are chewed by people in Serengeti national park in east Africa to prevent tooth decay. Plants produce more than 10,000 different compounds to prevent themselves against animals who feed on them. Almost half of all prescribed drugs contain chemicals produced by plants, fungi and bacteria. Aliphatic drugs also contain synthesized compounds in the laboratory that have been modeled after plants originating compounds (Moore et al., 1995).

The use of medicinal plants to treat diseases is as old as man. Medicinal plants have been used since ancient times to treat many illnesses (Mir et al., 2013). Research has shown that the concentration of these compounds in plants is directly related to their capability to treat certain illness. Many of these non-nutritive secondary metabolites are found in plants which are even used for food. Over 80% of the plants in Nigeria used for treatment of malaria and other sicknesses are also used as food (Cousins and Huffman, 2002); there seem to be not much distinction between medicinal benefits of plants and their nutritive value.

Over the past few years much research has been done and is still going on to prove scientifically the plants nutritional value and medicinal value. A good number of chemical compounds have been discovered from plants and found to have pharmacological value; this has led to the development of over 25% of all the artificial medicines used today. Many of the traditional plants species used all over the world have been found to have great pharmacological value. Studies carried out throughout Africa confirm that indigenous plants are the main constituents of traditional medicines. The published WHO traditional strategy addressed the issues and provided a framework for countries to develop policies to govern medicinal plants use. The strategy put forward by WHO advocates the formulation of a policy by states as the first component of developing traditional medicine. India is one of the few countries which have started to develop such policies (Prajapati and Purohit, 2003).

Over 80% of the people in developing countries use medicinal plants to treat the illnesses which affect them from time to time (Ganga et al., 2012). This can be attributed to poverty in these countries which has led to inefficient health care system in hospitals and inadequate resources to access these facilities. People in these countries look for cheap and available medicines which are known traditionally to cure the illnesses. The use of herbal medicines in the western world is steadily growing with 40% of the population using plants to treat illnesses; while in Kenya 90% of the population has one

time in their life used medicinal plants (Adongo et al., 2012). The use of these plants in treatment of ailments is mainly based on the type of flora in that region.

Our environment is very rich with a great range of medicinal plants and this mainly explains the reason why our grand's lived for quite some time. They could stay in the bush during war and even could use plants to treat ailments and wounds affecting soldiers in the battle ground. People all over the world should go back to these basics of treatment. Many communities in Africa still consider the use of medicinal plants as an important part of their culture, just to mention, the Maasai community in Kenya still value their culture very much, the Kalenjn community and their medicinal fermented milk which is prepared mainly from medicinal plants such as *Senna didymobotrya stem* which previous studies have shown this plant to have a great potential in treatment of diseases such as typhoid, diarrhea and food poisoning caused by *Salmonella typhi, E.coli and Bacillus cereus* (Ngule et al., 2013). The reason why herbal medicine still remains controversial is because of some greedy practitioners who want to become wealthy by pretending to know much about the treatment of every disease that clients complain about. This has led to administration of wrong drugs which do not cure a patient leading to death of the individual. Proper scientific evidence needs to be provided in order to create confidence in medicinal herbs. The increase of multi-resistant strains of bacteria calls for new discoveries of new classes of antibiotics that can clearly inhibit these resistant strains. This is the reason why much research should be turned to plants which have been used since ancient times to treat many diseases (Cousins and Huffman, 2002).

The non-nutritive plant components are referred to as phytochemicals, which can be divided in two major categories primary and secondary, with the primary constituting of carbohydrates, proteins and chlorophyll and the secondary consisting of tannins, alkaloids, saponins, steroids, flavonoids, terpenoids and anthroquinones (Maobe et al., 2013). The secondary metabolites help the plant survive in the environment by protecting them against predators but research has shown that these metabolites can be used to treat diseases in both animals and humans (Kokwaro, 2009). The antibacterial activity of plants has been closely associated with the presence of these important compounds in the plant. Plants antibacterial activity against various bacteria such as *B.cereus, Klebsiella sp*., *Streptococcus pyogenes* and *Proteus vulgaris* has been closely associated to the presence of phytochemicals in the plants (Swamy et al., 2013). Physiological activities of phytochemicals have been found to include cancer prevention, antibacterial, antifungal, anti-oxidative, hormone action and enzyme stimulation.

Natural bioactive compounds have been investigated in plants and their pharmacological effects analyzed. Secondary metabolites functions on growth,

photosynthesis and other important plant activities have not been discovered but their medicinal values have been identified in most of them (Ghasemzadeh and Ghasemzadeh, 2011). Phytochemicals have been used to a greater extend in Asia for various purposes such as treatment of diseases (Bodeker, 2000).

The lack of scientific knowledge on the phytochemical constituents, antibacterial, antioxidants and toxicological properties limits the use of traditional herbal medicine (Nyaberi et al., 2008). Phytochemicals can really improve the activity of the currently used drugs by acting as efflux of existing pump inhibitors. Many drug resistant microbes are emerging from time to time and causing the need to such for new antibiotics to kill and inhibit their growth. Phytochemicals have been associated with reduction of drug resistant forms of bacteria (Stauri et al., 2007).

A big percentage of plants in the savanna and semi-arid areas of east Africa where Kenya is located contains alkaloids which have been associated with increase in renal secretion when ingested, hence used as a diuretics and in the treatment of dropsy (Kokwaro, 2009). The use of alkaloids, saponins and tannins as antibiotics has been scientifically justified (Mir et al., 2013). Majority of the pharmacologically active chemical compounds were found mainly in ethanol extracts which is contrary to previous researches which had affirmed the traditional way of extracting these compounds using water (Iqbal, 2012).

According to Coopoosamy and Naidoo (2011), the plant *Tetradenia riparia* has great effect in the treatment against skin infections. The plant has also shown great potency in the treatment of chest diseases. According to Ndamane et al., (2013), the plant showed antibacterial activity against all the gram positive and gram negative bacteria it was tested against. In Kenya, the plant has a wide variety of uses depending on the region. The plant is used traditionally in the rift valley and western region to treat against stomach problems, wound infections, throat infections and also it is believed to treat have anti-cancer activity. The plant is grown by the local Nandi community in Kenya along farm edges and around their homes. The plant is greatly used by traditional practioners to treat various diseases; however, some of the practioners find it difficult to transfer this information to their clients even upon request for the information about the plant. This calls for scientific documentation to enable the transfer of knowledge about the plants use. The literature available is also contradicting and therefore the current study was not only done to give a scientific justification of the plants traditional use but also to compare the data obtained in this study with that in literature and provide a scientific view on the same.

MATERIALS AND METHODS

Sample Collection and Preparation

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton. The plant samples were collected and identified by a taxonomist in the University of Eastern Africa, Baraton. The samples were thoroughly mixed and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

Extraction procedure

Using electric analytical beam balance fifty grams of the powdered leaves of the *Tetradenia riparia* was placed in 1000 ml conical flask, methanol and water were then added in the ratio of 9:1 respectively until the leaves were completely submerged in the solvent. The mixture was then agitated for thorough mixing. The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman no.1 filter paper and a vacuum and pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary vacuum evaporator (R-11) with a water bath at 40^{o}C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and used for the experiment.

BIOASSAY STUDY

Preparation of the Bacterial Suspension:

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard, a procedure similar to that used by *Biruhalem et al., (2011)* and Donay *et al.,* (2007). The McFarland standard was prepared by dissolving 0.5 g of $BaCl_2$ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulphuric acid solution. Three – five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at 37^{0}C for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to $0.132A^{0}$ at a wavelength of 600 nm in order to obtain an approximate cell density of $1x10^{8}$ CFU/ml.

Preparation of the Extract Concentrations and Antibiotic:

Stock solutions for the extracts were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 500 mg of Penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Determination of bioactivity of the Extract:

Mueller Hinton agar plates were prepared by the manufacturer's instruction. The media was sterilized in an autoclave at 121^{0}C for 15 minutes. The plates were also sterilized at

the same temperature. The media was then poured on to the plates and air bubbles removed from the surface of the plates using non luminous Bunsen burner flame. The bacterial suspension was smeared on the surface of the plates using a sterile swab. Five wells were then drilled in each agar plate. Three of the wells were filled with the plant extract. The other wells were filled with penicillin and DMSO control respectively. Three plates were made for each bacterial organism and extract giving a triplicate reading for each microorganism and extract. The wells were labeled on the underside of the plate and incubated at 37^0C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a ruler.

RESULTS AND DISCUSION

Table 1: Zones of Inhibition (mean ± S.E.) of 500 mg/ml of *Tetradenia riparia* Extract against Selected Microorganisms.

Microorganisms	Mean ± S.E.	Penicillin	DMSO control
Escherichia coli	11.67±0.882	40.00±0.000	0.00±0.000
Salmonella typhi	0.00±0.000	31.00±0.000	0.00±0.000
Serratia liquefaciens	12.67±0.667	36.00±0.000	0.00±0.000
Enterobacter aerogenes	8.67±0.882	40.00±0.000	0.00±0.000
Bacillus cereus	28.00±1.154	45.00±0.000	0.00±0.000
Proteus vulgaris	11.00±0.577	46.00±0.000	0.00±0.000

dimethylsulfoxide (DMSO)

The average mean zone of inhibition (±S.E.) was calculated for each of the microbial organism. The zones of inhibition of the microorganisms were also analyzed by analysis of variance (ANOVA) and it was shown that there were significant differences in the zones of inhibition among the microbial organisms ($p<0.05$). The biggest zone of inhibition was against *B. cereus* (28.00±1.154) followed by *S. liquefaciens* (12.67±0.667), *Escherichia coli* (11.67±0.882), *Proteus vulgaris* (11.00±0.577) and *Enterobacter aerogenes* (8.67±0.882). Penicillin which was used as the positive control inhibited the growth of all the organisms while DMSO did not have any inhibition against the organisms tested.

Table 2: Turkey's Honestly Significant Difference among Microorganisms. (Using 500mg/ml of *Tetradenia riparia* Extract)

Comparison	P Value	Significance
E. coli vs *Salmonella typhi*	0.001	S
E. coli vs *S. liquifaciens*	0.995	NS
E. coli vs *E. aerogenes*	0.084	NS
E. coli vs *Bacillus cereus*	0.000	S
E. coli vs *Proteus vulgaris*	0.999	NS
Salmonella typhi vs *S.liquefacines*	0.000	S
Salmonella typhi vs *E. aerogenes*	0.111	NS
Salmonella typhi vs *B. cereus*	0.000	S
Salmonella typhi vs *P. vulgaris*	0.001	S
S. liquefaciens vs *E. aerogenes*	0.036	S
S. liquefaciens vs *B. cereus*	0.000	S
S. liquefaciens vs *P. vulgaris*	0.953	NS
E. aerogenes vs *B. cereus*	0.000	S
E. aerogenes vs *P. vulgaris*	0.145	NS
B. cereus vs *P. vulgaris*	0.000	S

The study shows that the plant *Tetradenia riparia* can inhibit the growth of five microorganisms out of the six it was tested against. The plant show clear zones of inhibition against *Escherichia coli* with a zone of inhibition of 11.67 ± 0.882, *S. liquefaciens* 12.667 ± 0.667, *Bacillus cereus,* 28.00 ± 1.154, *Enterobacter aerogenes,* 8.67±0.882 and *Proteus vulgaris,* 11.00 ± 0.577. Comparing for the mean zones of inhibition of 500 mg/ml of *Tetradenia riparia* showed that there was significant difference in the zones of inhibition among the organisms ($p<0.001$). Further comparison using the Tukey's pairwise comparison showed that the zones of inhibition for *E. coli* were significantly higher than those of *Salmonella sp.* and *P. vulgaris* ($p<0.05$). Zones of inhibition of *Salmonella typhi* were significantly lower than all of the other organisms ($p<0.05$) except for *E. aerogenes*. Significant difference were also observed between *B. cereus* and all the organisms ($p<0.05$). The study is in conformity with previous studies in which the plants methanol and water extracts were found to inhibit the growth of all the gram positive and gram negative bacteria (Ndamane et al., 2013), The study is completely in agreement with the same study in which the plant inhibited *B. cereus* most. The current study use a system of solvent which constituted methanol and water in the ratio of 9:1 unlike in previous studies the two were used separately. The data obtained in this study in conformity with previous studies which shows the two solvents to have great antibacterial activity, however, contradicts with the results obtained by Erasto et al.,

(2005), the plant did not show antibacterial activity against *E. coli* and *B. cereus*. The antibacterial activity of the plant can be attributed to the presence of the phytochemicals. The phytochemicals found in the plant have been investigated and found to have antibacterial activity therefore justifying traditional plant use to treat against various diseases caused by bacteria (Gazim et al., 2010).

The plant extract can be used to treat infections caused by *Bacillus cereus* viz posttraumatic wounds, self-limited gastroenteritis, burns, surgical wounds infections, and ocular infections such as endophthalmitis, corneal abscess and panophthalmitis (Garcia-Arribas et al., 1988) & Sankararaman and Velayuthan, 2013). The plant extract can also be used to treat immunologically compromised patients including AIDS and malignant disease victims (Cotton et al., 1987 & Tuazon et al. 1979). The plant's ability to inhibit the growth of *E. coli* is a scientific justification that the plant can be used to treat against enteric infections caused by the bacteria. The plants extract can also be used to treat against gastro-intestinal diseases, ear infections, urinary tract infections and wounds infections caused by *Proteus vulgaris (*Goodwin et al., 1971 & Neter and Farrar, 1943).

Indigofera arrecta can be a good source of active compounds for a variety of diseases affecting human beings in the world today. The plants ability to inhibit the growth of *Serratia liquefaciens* shows how the plant can be important to treat against the bacteria which according to Okunda et al., (1984) cause nosocomial urinary tract infections. The inhibition of the plant against these bacteria is, therefore, note worthy since the microorganisms have been found to have resistance against most of the currently used antibiotics. *Enterobacter aerogenes* is a major cause of a wide variety of nosocomial infections viz, pneumonia, urinary tract infections, meningitis, wound infections and intravascular and prosthetic devices infections (Santos et al., 1990, Blot et al., 2003, Donnenberg, 2005).

CONCLUSION

The data provided in this study is a scientific justification that the plant *Tetradenia riparia* can be used to treat against diseases such as abdominal cramps and diarrhea caused by *Bacillus cereus* due to its ability to cause food poisoning, treat against *Escherichia coli* which causes serious and even life threatening effects such as hemolytic-uremic syndrome (HUS), diarrhea and neonatal meningitis. It can also be used to treat against throat problems caused by *Serratia liquefaciens*, opportunist pathogen *Proteus vulgaris* which causes wound infections. From the study the plant *Tetradenia riparia* has shown to have great medicinal value and therefore justifying its traditional use to treat against various diseases. More research needs to be done to identify the mode of action of the active compounds in the plant.

ACKNOWLEDGEMENTS
The authors of this paper are very much thankful to the Department of Chemistry and Medical Laboratory Science, University of Eastern Africa, Baraton. Authors are also thankful to taxonomist Mr. Joel Ochieng Ondiek, University of Eastern Africa, Baraton for his great assistance in the identification of the plant.

REFERENCES

Adongo, S.O., J.Morongo, R.Anjou and F. New (2012). Analysis of selected essential elements of medicinal plants used by Chuka community, Tharaka Nithi county, Kenya. *The Scientific Journal of Science and Technology*, Special issue 87-94.

Anthoney Swamy T, C.M. Ngule and O. Jackie (2013). Phytochemical Analysis of *Vernonia adoensis* Leaves and Roots Used as a Traditional Medicinal Plant in Kenya. *International Journal of Pharmacy and Biological Sciences*, 3 (3) 46-52.

Biruhalem, T., M. Giday, A.Animut, J.Seid (2011). Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pacific Journal of Tropical Biomedicine,* 370-375.

Blot, S.I., Vandewoude, K.H., Colardyn, F.A. (2003). Evaluation of outcome in critically ill patients with nosocomial *Enterobacter* bacteremia: results of a matched cohort study. *Chest*. 123:1208-13.

Bodeker G. (2000). Traditional health system: valuing biodiversity for human health and wellbeing. *In cultural and spiritual values in biodiversity, ed. D.A Posey,* 261-284. Nairobi: practical action.

Coopoosamy, R.M., K.K.Naidoo (2011). Assessing the potential of Tetradenia riparia in treatment of common skin conditions in rural communities of South Africa. *Afr. J. Microbiol. Res*. 5(19):2942-2945.

Cotton, D.J., Gill, V.J., Marshall, D.J., Gress, M., Thaler, M. and Pizzo, P. (1987). Clinical features and therapeutic interventions in 17 cases of *Bacillus bacteremia* in an immunosupressed patient population. *J. Clin. Microbiol*, 25: 672-674.

Cousins D and A.M.Huffman (2002). Medicinal property in the diet of gorillas: Ethno-pharmacological evaluation, 23(2), 65-89.

Donay, J.L., P.Fernandes, P.H.Lagrange, J.L.Herrmann (2007). Evaluation of the inoculation procedure using a 0.25 McFarland Standard for the BD Phoenix Automated Microbiology System, *Journal of Clinical Microbiology*, 45 (12), 4088-4089.

Donnenberg, M.S. *Enterobacteriaceae*. In: Mandell GL,Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's. (2005). Principles and practice of infectious diseases. 6th ed. Philadelphia: Churchill Livingstone, 2579.

Erasto, P., P.O.Adebola, D.S.Grierson, A.J.Afolayan (2005). An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province South Africa. *Afr. J. Biotechnol,* 2005, 4(12):1458-1460.

Ghasemzadeh A. and N. Ghasemzadeh (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*, 5 (31), 6697-6703.

Ganga, R.B. V.Y. Rao, V.S.P. Pavani (2012). Quantitative and qualitative phytochemical screening and in vitro antioxidant and antimicrobial activities of *Elephantopus Scarber* Linn. *Recent Research in Science and Technology*, 4(4), pp 15-20.

Garcia-Arribas, M.L., Plaza, C.J., De La Rose, M.C. and Mosso, M.A. (1988). Characterization of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. *J. Appl. Bacterial,* 64: 257-264.

Gazim, Z.C., C.L.Ana, M.C.Ana, M.R. Claudia, et al. (2010). Seasonal variation, chemical composition, and analgesic and antimicrobial activities of the essential oil from leaves of *Tetradenia riparia* (Hochst.) codd in Southern Brazil. *Molecules*, 15: 5509-5524.

Goodwin, C.S., Kliger, B.N., and Drewett, S.E. (1971). Colistin-sensitive Proteus organisms: Including indole-negative *Proteus vulgaris*, non-swarming on first isolation. *Br. J. exp. Path.*, 52: 138-141.

Iqbal J.P. (2012). Phytochemical screening of certain plant species of Agra City. *Journal of Drug Delivery and Therapeutics*, 2(4), 135-138.

Kaoli K. V. and V. K. Kauli (2011). Review on pharmaceutical properties and conservation measures of *Pontellafulgens* wall. Ex-Hook – A medicinal endangered herb of higher Himalaya, 2 (3), 298-306.

Kokwaro J.O. (2009). *Medicinal plants of East Africa*. Nairobi: University Press.

Ndamane, Y., L. Kambizi, S.P.Songca, and O.S.Oluwafemi (2013). Antibacterial effectiveness of *Tetradenia riparia* extract, a plant traditionally used in the Eastern Cape Province to treat diseases of the respiratory system.*Journal of Medicinal Plants Research*, 7(37): 2755-2760.

Neter, R.E. and Farrar. H.R. (1943). *Proteus vulgaris* and *Proteus morganii* in diarrhea disease of infants. *The American Journal of Digestive Diseases,* 10(9): 344-347.

Ngule, C.M. Anthoney Swamy T and J. K. Obey (2013). Phytochemical and bioactivity evaluation of *Sennadidymobotrya*fresenirwin used by the Nandi community in Kenya. *Int. J. Bioassays*, 2 (07), 1037-1043.

Njoroge, G.N. and R.W. Bussman (2006). Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyu's (central Kenya). *Journal of ethnopharmacology,* 108 (3): 332-339.

Nyaberi, M.O., C. A. Onyango, F.M. Mathook, J. M. Maina, M. Makobe and F. Mwaura (2008). Senna didymobotryafresenirwin and Barneby used by the pastoral communities in west pokot to preserve milk. *Natural resource management Kenya*, PP 980-986.

Maobe, M.A.G., E. Gatebe ,L. Gitu and H. Rotich (2013). Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, southwest Kenya. *European Journal of Applied Sciences,* 5(10) 01-06.

Mir, M.A, S.S.Sawhney and M.M.S.Jassal (2013). Qualitative and quantitative analysis of phytochemical of *Taraxacumofficinale*. *Wud Pecker journal of pharmacy and pharmacology*, 2 (1), 1-5.

Moore, R. D.W. Clark, R.K.Stern, and D.Vodopich (1995). *Botany*. USA: WMC. Brown Communications, Inc,.

Okuda T., Endo N., Osada Y and Zen-Yoji H. (1984). Outbrea of nosocomial urinary tract infections caused by *Serratia marcescens.* Journal of Clinical Microbiology, 20(4): 691-695.

Prajapati D.N. and S.S. Purohit (2003). *Agro's color atlas of medicinal plants*. Agrobios India: New Delhi.

Sankararaman S. and Velayuthan, S. (2013). *Bacillus cereus*.Peditrics in Review, 34: 196.

Santos Filho, D., Sarti, S.J., Bastos, J.K., Leitao Filho, H.F., Machado, J.O., et al. (1990). Atividade antibacteriana de extratos vegetais. *Rev. Cien.Fam*, 12: 39-46.

Stauri, M., J.U.L.Piddock & S. Gibbans (2007). Bacterial efflux pumps from natural sources. *Journal of Antimicrobial Chemotherapy*, 59, 1247-1260.

Tuazon, C.U., Murray, H.W., Levy, C., Solny, M.N., Curtin, J.A. and Shegren, J.N. (1979). Serious infections from Bacillus species. *JAMA*, 241:1137-1140.

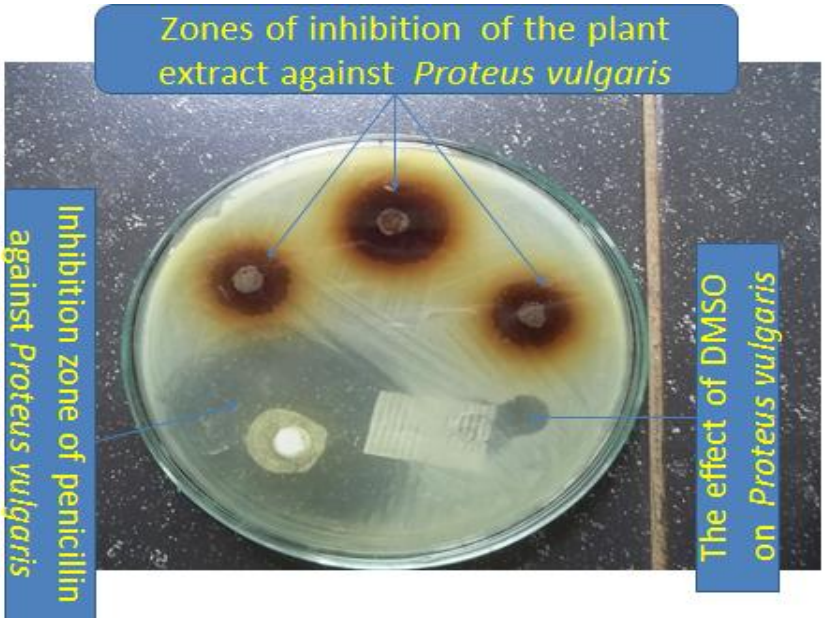


Fig 1. Picture showing the zones of inhibition of the plant extract and positive and negative action against *Proteus vulgaris*

Fig 2. *Tetradenia riparia* plant leaves

CHAPTER FIVE

MEDICINAL PROPERTIES OF INDIGENOUS PLANTS USED IN THE PREPARATION OF TRADITIONAL RICE BEVERAGE "*HANDIA*" AMONG THE TRIBALS OF MAYURBHANJ DISTRICTS, ODISHA, INDIA

Sujogya Kumar Panda[1], Laxmipriya Padhi[1] and Akshaya Kumar Bastia[2]
[1]Department of Zoology; North Orissa University; Baripada, Odisha, India-757003
[2]Department of Botany; North Orissa University; Baripada, Odisha, India-757003

ABSTRACT

The district Mayurbhanj is charecterised by a diverse tribal population of people with different ethinic background. Similipal Biosphere Reserve is present at the center of Mayurbhanj District; its rich biodiversity is acknowledged internationally. The district as a whole and SBR in particular, offers unique opportunities to study the indigenous knowledge and their uses prevalent among the local tribes. The native tribals depend on wild as well as cultivated plants for their various food and medicinal purposes. As a part of the socio-cultural life, all tribes prepare rice beverage using their own unique starter culture. In the preparation of starter they use some of the wild plants as antimicrobials without knowing the actual role of these plants in fermentation. They say that yeast is formed from these plants, responsible for yeast action during the fermentation. This chapter deals with the available plants used for starter preparation of Handia by the tribes of Mayubhanj, their ethnomedicinal uses. All these plants were evaluated for the phytochemical constituents and antibacterial activity against enteric pathogens. Results found that alcoholic extracts of the plants contain abundant alkaloids, flavonoids, carbohydrate, protein and amino acids, saponins, tannin and phenolic compounds. With the exception of Aspargus racemosus (root), Cissampelos pareira (leaf), Dioscorea sp. (tuber), Rauwolfia serpentina (leaf) extracts, all test plant parts exhibited antibacterial activity by agar cup method. The zone of inhibition was found maximum against Staphylococcus aureus followed by Shigella sonnei and S. flexneri. The MIC result ranged from 125 to 1000 µg/ml (w/v) with the lowest against S. aureus (125, 156, 250 µg/ml) followed by S. sonnei (156, 250, 312, 500, 625 µg/ml). MBC test validate that in between 1000-2500 µg/ml (w/v) concentrations, most of test bacteria were killed due to broad spectrum activity. This chapter also provides information on different types of starter used for preparation of rice beverage through out India.

KEYWORDS: *Bakhar, Alcoholic beverage, Traditional knowledge, Process technology, Ethnomedicine, Enteric pathogens*

INTRODUCTION

A wide range of cereal based fermented foods exist throughout the Asian and African countries. Since rice is the major cereal in these areas, a global interest in rice and its fermented product is increasing due to their caloric value, unique quality characteristics and high acceptability (Steinkraus, 1994). In most of the countries, rice is fermented either by using mixed culture(s) into alcoholic beverages, or by natural fermentation into leavened batter formed dough breads which are usually baked or steamed (Yokotsuka, 1991). Rice beer is an integral part of life of several aboriginal communities throughout the world. In India, from time immemorial, both fermented and distilled beverages have been prepared by fermenting different varieties of rice. These beverages are primarily prepared and used by different tribal communities of the northern and eastern part of India.

In Odisha, from time immemorial, both fermented and distilled beverages have been prepared by fermenting different varieties of rice. These beverages are primarily prepared and used by different tribal communities all over the state. About 62 ethnic tribal communities are reported from the state (Naik, 1998) and they mostly inhabit forest villages. They meet most of their requirements, including food and primary healthcare, from the forest resources. Out of 62, 30 communities (48%), several aboriginals, are found in the district of Mayurbhanj, (largest district of the state; area - 10, 418 sq km forest cover – 4, 392 sq km. population – 25, 13, 895/2011 census). Similipal Biosphere Reserve (SBR, 5569 sq. km.) is located at the centre of the district and its rich biodiversity is acknowledged internationally. The district as a whole and SBR in particular, offers unique opportunities to study the indigenous knowledge and their uses prevalent among the local tribes. *Santal, Kolha, Bathudi, Bhumij, Munda* and *Gond* are major tribes whereas *Mankidia, Lodha, Kisan* and *Baiga* are minor tribal groups that inhabit the area. *Santals* constitute the largest tribal group of the district and are scattered throughout. The social, cultural and religious life of aboriginal people is influenced by the nature and natural resources available in and around their habitat that provide food, fodder, medicine, shelter and various other material and cultural needs.

The fermented food, locally known as *handia*, is an inseparable food item in the life of tribals of Mayurbhanj and most other districts of the state. The word *handia* finds its origin from the word *Handi* in *Odia* (local language), means large earthen pot. *Handia* occupies a key position in the social, cultural and economic life of *Santals* and accepted as a traditional drink (Sahu, 1996). *Handia* is generally prepared throughout the year, but most common during the summer months (March to June). Women and children are also fond of these beverages but consume in small quantity and preferably during festivals, ceremonies and on Sundays. Tribals get 5-10% of their daily nutrient requirements that plays a supplementary role in the nutrition of the people (Roy, 1978).

It is prepared from rice along with some of the locally available plant parts through some indigenous method. Otherwise known as country liquor or poor man's whiskey, it is relished by one and all and in most occasions. The process of starter (locally known as Bakhara/Ranu) preparation of each tribe is almost similar with slight differences. The main ingredients of starter preparation are rice along with some of the locally available plant parts through some indigenous method. So, a survey of the use of medicinal plants in preparation of Bakhara/Ranu by the tribes in the District Mayurbhanj was carried out. The tribals do not know the authentic role of these plants in the fermentation. According their knowledge, either yeast is formed from these plants or these plants are responsible for the yeast's action in fermentation. The present paper deals with the description and ethnomedicinal uses of plants for the starter preparation by the tribes of Odisha. For scientific validation all these plants were subjected for screened of antimicrobial activity and phytochemical screening.

LITERATURE REVIEW

Traditional rice-based alcoholic beverages

Traditional rice beverages have different compositions according to the formulation and processes used. The principle of their manufacture can be characterized as a biochemical modification of cereal starches brought about by microorganisms. Moulds produce the amylases that degrade the starch into dextrins and sugars and yeasts convert these sugars to alcohol (Lim, 1991; Motarjemi and Nout, 1996; Nout and Aidoo 2002).

The preparation and the use of fermentation starters as a source of inoculum are important for preparation of rice beverage. The choice of starter tablets influences the yield and quality of the rice beer. Even in certain region the local processors claim that using a combination of two or three different starters yields better quality with a stronger sweet alcoholic taste and more attractive flavor than is obtained with a single starter. The dried starters normally include yeasts, moulds and bacteria and convert starchy materials to fermentable sugars and subsequently to alcohol and organic acids (Hesseltine *et al.*, 1988; Nwosu and Ojimelukwe, 1993; Luong, 1998; Nout and Aidoo, 2002). A variety of starter cultures is available in the through out India especially in the North East region (Table-1).

Table-1: Starters for alcoholic beverages used by different tribes of India

Product	Plants used during preparation	Functional moulds, yeasts and bacteria
Angkur	*Xanthium strumarium, Scoparia dulcis* L., *Clerodendrum viscosum* L.	Un known
Aopo pitha	*Achyranthes aspera* L., *Cinnamomum bejolghata, Adhatoda Vasica* Nees., *Ageratum conyzoides* L., *Ananas comosus* (L.) Merr., *Artocarpus heterophyllus, Asparagus racemosus* Willd., *Cinnamomum tamala* Nees., *Capcicum annum* L., *Centella asiatica* L., *Clerodendrum viscosum* L., *Costus speciosus* (Koen. ex. Retz.) J.E. Smith, *Drymeria cordata* L., *Gomphostemma parviflora* Wall., *Ipomoea aquatica* Forsk, *Ipomea mauritiana Jacq., Kaempferia rotunda* L., *Leucas plukenetii* (Roth) Spreng., *Lygodium flexuosum, Melothrea heterophylla* (Lour) Cogn, *Microsorum punctatum* (L.) Copel, *Musa balbisiana Colla, Naravelia feylavica* (D.C), *Oldenlandia corymbosa* L., *Piper longum* L., *P. nigrum* L., *Phlogacanthus thyrsiformis* (Hardw.) Mabb., *Psidium guajava* L., *Pueraria tuberose* (Roxb. ex. Willd.) DC, *Ptridium aquilinum* Kuhn, *Saccharum officinarum* L., *Scoparia dulcis* L., *Selaginella* species, *Swernia chirata* (Buch-Hem), *Vitex negundo* L., *Zanthoxylum nitidum* (Roxb.) (Gogoi *et al.,* 2013; Das *et al.,* 2012)	Un known
Bakhar	*Heteropogon contortus* (L.) Beauv. ex R. & S., *Bambusa vulgaris* Schrad,	*Mucor* sp., *Rhizopus* sp., *Amylomyces* sp., Yeast
Epop	*Scoparia dulcis* (Linn.); *Pteridium* sp; *Adhatoda vasica* (Nees); *Cyclosorus* sp., *Adhatoda zeylenica, Costus speciosus, Naravelia zeylanica, Melothrea heterophyla; Zanthoxylum hemiltonianum* (Wall); *Phogocanthus thyrsiflorus* (Nees); *Hydrocotyl rotundifolia* (Roxb); *Centella asiatica; Swertia chirata* (Buch-Hem); *Actinodaphne obovata* (Blume); *Piper nigrum* (Linn); *Piper longum* (Linn); *Selaginella* sps., *Lygodium japonicum* (L); *Naravelia feylavica* (D.C.); *Melothrea heterophylla* (Lour) Cogn	Un known

Hamei	*Albizia myriophylla* Benth.	*Mucor* sp. And *Rhizopus* sp.; *Saccharomyces cerevisiae*, *Pichia anomala*, *P. guilliermondii*, *P. f abianii*, *Trichosporon* sp., *Candida tropicalis*, *C. parapsilosis*, *C. 43ontana*, and *Torulaspora delbrueckii*; *Pediococcus pentosaceus* and *Lactobacillus brevis* (Tamang et al. 2007a; Jeyaram et al. 2008b; Singh and Singh, 2006).
Humao/ Umhu	*Glycyrrhiza glabra* L. (Chakrabarty *et al.,* 2009) *Acacia pinnata* (Das *et al.,* 2012)	*Saccharomyces cerevisiae*
Khekhrii	*Lagenaria siceraria* [Molina] Standley; *Elscholtzia blanda* Benth., *Elscholtzia blanda*, *Justicia adhatoda* Nees. (Tamang, 2010)	*Saccharomyces cerevisiae* (Teramoto *et al.,* 2002; Sekar and Mariappan, 2007)
Marcha	*Plumbago zeylanica*, *Buddleja asiatica*, *Vernonia cinerea*, *Polygala arillata*, *Clematis grewiaeflora*, *Polygala* sp., *Polygala arillata*, *Piper chaba*, *P. longum*, *Christella appendiculata*, *Elephantopus scaber*, *Inula* sp., and *Scoparia dulcis* (Tamang, 2010)	*Rhizopus* sp., *Mucor* sp., *Mucor circinelloides*, *M. hiemalis*, *Rhizopus chinensis*, and *R. stolonifer* var. *lyococcus*; *Endomycopsis fibuliger. Saccharomycopsis fibuligera*, *Saccharomycopsis capsularis*, *Pichia anomala*, *P. burtonii*, *Saccharomyces cerevisiae*, *S. bayanus*, and *Candida glabrata*; *Pediococcus pentosaceus*, *Lactobacillus bifermentans*, and *Lb. brevis* (Tamang 1992; Tamang and Sarkar 1995; Thapa 2001; Tsuyoshi et al. 2005; Tamang, 2010).
Mod pitha	*Allium sativum* L., *Artocarpus heterophyllus* Lamk., *Ananas comosus* (L.) Merr. *Alpinia malaccensis* Rosc., *Alternanthera sessilis* (L.) R. Br. ex DC., *Capsicum annuum* L., *Cinnamomum bejolghota* (Buch.-Ham) *Sw*., *Centella asiatica* (L.) Urban, *Coffea bengalensis* Roxb., *Costus speciosus*	Un known

	J. E. Sm., *Desmodium* sp., *Cyprus* sp., *Desmodium pulchellum* (L.) Benth. *Equisetum* sp., *Lygodium flexuosum* (L.) Sw., *Melastoma malabathricum* L. *Mussaenda roxburghii* Hook.f., *Myxopyrum smilacifolium* (Wall.) Bl., *Naravelia zeylanica* (L.) DC., *Oryza sativa* L. *Psidium guajava* L., *Pothos scandens* L., *Pteridium aquilinum* (L.) Kuhn., *Pycnarrhena pleniflora* Miers., *Rubus* sp., *Saccharum officinarum* L., *Selaginella semicordata* (Wall) Spreng, *Scoparia dulcis* L., Solanum torvum Sw., *Thunbergia grandiflora* Roxb., *Zanthoxylum oxyphyllum* Edgw., *Zingiber officinale* Rosc. (Deori *et al.,* 2007)	
Perok Kushi	*Jasminum sambac*; *Cinnamomum byolghata*; *Zanthoxylum hamiltonianum*; *Lygodium flexuosum*; *Acanthus leucostychys*; *Cyclosorus exlensa*; *Alstonia scholaris*; *Alpinia malaccensis*; *Costus speciosus* (Das *et al.,* 2012)	Un known
Ranu dabai	*Oryza sativa* L., *Coccinia grandis* L., *Vernonia cinerea* L., *Clerodendrum viscosum* Ventenat,, *Plumbago zeylanica* L.,, *Stephania japonica* (Thunb.)Miers,, *Stephania glabra* (Roxb.) Miers,, *Oroxylum indicum* L.,, *Mussaenda roxburghii* Hook.f.,, *Scoparia dulcis* L.,, *Rauvolfia serpentina* L.,, *Artocarpus heterophyllus* Lam., *Wattakaka volubilis* (L.f) Benth	Several mycelia fungus and Yeast
Ranu goti/ tablets	*Argyreia bella* (C.B.Clarke) Raizada, *Bombax ceiba* L., *Buchanania lanzan* Spreng., *Casearia graveolens* Dalz., *Cassine glauca* (Rottb.) O. Ktze, *Catunaregam spinosa* (Thunb.), *Cissampelos pareira* L., *Crotalaria albida* Heyne ex. Roth, *Cryptolepis buchanani* Roem. & Schult., *Datura metal* L., *Elephantopus scaber* L., *Euphorbia prolifera* Buch.-Ham. ex D. Don, *Hemidesmus indicus* (L.) R. Br., *Holarrhena pubescens* Wall. ex Don, *Knoxia sumatrensis* (Retz.) DC., *Pueraria tuberosa* (Willd.) DC., *Scoparia dulcis* L., *Senecio nudicaulis* Buch.-Ham. ex D. Don, *Symplocos racemosa* Roxb., *Tylophora rotundifolia* Buch.- Ham. ex. Wt., *Wattakaka volubilis* (L.f.) Stapf (Kumar and Rao, 2007)	Several mycelia fungus and Yeast

Siiyeh/ Siye/Op op/Ipof	*Clerodendrum viscosum* L., *Veronia* sp. (Das *et al.,* 2012)	Un known
Thiat	*Amomum aromaticum* Roxb.	Yeast
Thap	*Croton joufra*, *Artocarpus heterophyllus*, *Phlogocanthus thysiflorus*, *Solanum viarum* and *Acacia pennata* (Das *et al.,* 2012)	Un known
Vekur pitha	*Lygodium flaxuosum* Linn., *Leucas aspera* Spreng, *Cissampelos pereira*, *Scoparia dulsis* Linn., *Centella asiatica; Cinamommum glanduliferum* Meissn., *Piper betle* L., *Piper nigrum* L., *Hydrocotyle sibthorpioides* (Das *et al.,* 2012)	*Saccharomyces cerevisiae*

Starter preparation and Alcoholic fermentation

The raw ingredients for the preparation of starter tablets can be either rice flour or cassava flour or combinations of rice flour and cassava flour. Also mixed flours are preferred in certain region. These mixtures are ground and thoroughly mixed with spices and herbs that are believed to play a role in preventing the growth of undesirable microorganisms. The spices and herbs used include mixtures of garlic, pepper, onion, rhizomes and root of oriental herbs and producers jealously guard their own secret recipes. The ratio of ground rice to mixed spices is different about 14:1 by weight. Water is added to form a dough-like mass with moisture content of 55-60% which is inoculated with dry powdered starter from previous batches, followed by thorough mixing. The inoculated dough is shaped into small flattened or ball-shaped cakes about 4 cm in diameter and 1cm thick. The cakes are placed on a bamboo tray and are then covered with a thin layer of rice husks. According to producers this reduces overheating and facilitates aeration. The tray is covered with a cloth and incubated in a ventilated place at ambient temperature (approx. 28-32°C) for 2-5 days during which time the dough rises slightly and becomes covered with fungal mycelium. The cakes are air or sun-dried and then have a shelf life of several months.

Throughout the world three different kinds of starters are used e.g. starters without oriental medicinal herbs; starters supplemented with oriental medicinal herbs; and starters supplemented with plant containing aromatic essential oils. In the most cases, starters supplemented with medicinal plants are predominating. It was suggested by some authors that a number of plants have a stimulatory effect on biomass and on yeast count. Few authors believe that plants are used in starter because of their antibacterial properties and their fragrant flavour.

Preparation of *Angkur* by the *Bodos* tribes of Assam (Das *et al.*, 2012)

Jou Bishi is the local rice beer prepared by the *Bodos* using the starter cakes known as *angkur*. For preparing *angkur*, different plant materials are said to be used based on their availability in different regions. The most common species are leaves of *Xanthium strumarium*, *Scoparia dulcis* and either roots or leaves of *Clerodendrum viscosum*. These plants are first washed properly and allowed to dry in the air. Rice grains are soaked for about 5 hours in normal temperature water and allowed to soften. This is then mixed with the plants and grinded together in a wooden mortar with a pestle and this set of apparatus is called *wayal*. Dough is made by adding a little water to the mixture. They are then made into round cakes with different size and covered with powder of the mixture to which water is not added. This is followed by covering with *gigab* (paddy straw) and allowed to dry for a period of 3-4 days. These can be stored in moisture free places for more than a year.

Preparation of *Apop Pitha* by the *Mising* tribes of Upper Assam (Gogoi *et al.*, 2013)

For preparing apop pitha a mixture of plants (table) collected, cleaned and dried by placing on a bamboo mat. Soaked rice and the leaves are grinded separately in a wooden grinder and they are mixed together in a vessel with a little of water. From the dough, oval shaped balls of about (6 cm x3cm) are made and dried in the sun. Apop pitha is used for preparation of Apong-rice beer. The earthen pot is use as a fermentor and before starting the fermentation process, it is fumigated by placing it on a bamboo frame constructed over the fire place until the pot turns blackish. There after the boiled rice are spread over a large banana leaf and allowed to cool. To this powdered *apop pitha* is added (1 *apop pitha* for 1 kg of rice) and the whole mixture is kept inside the fermentor and the mouth of the pot is covered with banana leaves or leaves of *bhilongoni* (*Cyclosorus exlensa*). The fermentor is left for a period of at least 5 days. A little water is added to the fermented product and is filtered to get the *apong* (Das *et al.*, 2012). Similar type of starter is also prepared by the Khampti tribe of Arunachal Pradesh locally known as Khamtip for preparation of Apong (Srivastava *et al.*, 2012).

Preparation of *Bakhor, Surachi/Phap* by the *Rabha* tribes, Assam (Deka and Sharma, 2010)

Rice-beer cake is popularly known as *bakhor, surachi* or *phap* among the Rabhas of Goalpara district while rice-beer is known as choko or jongamod. Paste of 2 kg wet seeds of rice (*Oryza sativa* L.) is prepared after mixing several plants. A considerable amount of old rice-beer cake is mixed along with these plant materials for the preparation of fresh rice-beer cakes. Some round and flat globules (each of around 50 gm) are prepared from the grinded mixture. The globules are placed on straw. Different parts of ten plant species are used in particular amount to prepare rice-beer cake. *Heteropogon contortus* is kept scattered on a broad sieve made of bamboo (*Bambusa*

vulgaris) and then sun dried. To prepare rice-beer choko or phap, tightly cooked fresh rice (using less amount of water) is used. After cooking, Rabhas scatter rice on a broad mat made of bamboo and cooled. Then a particular amount of rice beer cake (generally 2 pieces for boiled rice prepared from 2 kg of fresh rice) is powdered and mixed with the cooked rice. A special type of cylinder made of bamboo net, known among Rabhas as janthi, is placed inside an earthen pitcher, known as jonga. Now, already prepared mixture of rice and rice-beer cake is kept inside jonga and outside janthi. At last, open mouth of jonga is tightly sealed with banana (*Musa balbisiana*) leaf warmed in fire and placed in a dark place. Rabhas place *Ricinus communis* L. leaf and one piece of wood charcoal over the lid of jonga made of banana leaf ward off the effect of evil sprit. During summer, (after 4-5 days) and during winter (after 7-8 days), choko or rice-beer attains the actual stage for drinking. A hole is made at the venter of dried shell of matured fruit of *Lagenaria siceraria* Standl. and used to collect the local alcoholic drink stored inside janthi placed inside jonga. The rice-beer prepared through the above process of fermentation is again fermented adding particular amount of water and rice-beer cake. After 3-4 days, rice beer is collected and distilled through a local process using 3 earthen or metalic pitcher-like pots (hadi / luduki), placing one over another; 2nd and 3rd pots having a hole at the bottom. The whole equipment is made air-tight using jute (*Corchorus capsularis* L.) fibre and mud at the junctions. The resulting drink is strong liquor, known as fotika and Rabhas believe that it has curative effect on psychiatric patients.

Preparation of *epop* (starter culture) by *Mishing* tribe of Assam (Kardong *et al.*, 2012)
The epop is prepared from rice, preferably glutinous, soaked in water for about 2 hrs., ground together and mixed properly with semidried powder of leaves or the whole plant/plant parts which is then kneaded to specific shape (usually oval) followed by the transfer (inoculation) of microbial consortium with desired quality from the old stock of previous batch culture. The preparation of epop and the poro apong is somewhat similar with babud preparation reported from Phillipines and the Ipoh preparation by Adi tribe of Arunachal Pradesh, India (Tiwari and Mohanta, 2007). Mishing tribe generally uses at least 16 different herbs with different interpretations (Table-1). This number may vary from place to place, persons to persons within the tribe itself.

Preparation of *Hamei* as a starter by Meitei tribes, Manipur (Singh and Singh, 2006)
Yu is a distilled product of the fermented local rice of Meitei communities of Manipur. For quality and more alcohol production of Yu, *Hamei* (a fermented product) is added generally, because of its action as a starter/catalyst. Moreover, the preparation of *Hamei* is a popular domestic business for the people of scheduled caste and tribes, as it is also used as an ingredient of cattle foods. The traditional practitioners believe that, the quality of *Hamei* fermentation will be responsible for the quality and quantity of ethyl alcohol.

Hamei has different name viz. *Andro, Sekmai, Phayeng, Jiribam, Bishenpur, Tengnoupal* etc and prepared using similar ingredients and methods except with the slight differences in shape, size and coverings during the process of fermentation. White rice of about 3 kg was pre-soaked for about half an hour and dried for 15 min to remove excess water. The white rice is prepared traditionally by pounding in a wooden mortar *(Shumban)* with a wooden mallet *(Shuk)* and the powder mass thus obtained is called *Yam*. Finely chopped or powdered about 250-300 gm dried bark of *Yanglee* (*Albizia myriophylla* Benth.) plant is mixed with required amount of water and filtered. The filtrate obtained appears brownish in colour. A homogenous mixture paste is prepared by mixing *Yam* and *Yanglee* filtrate. From this paste mass, a cake like structure in the form of elliptical or rounded flattened mass is prepared known as *Hamei*. Pressing a small portion of paste mass in between the palms does the preparation of *Hamei* cake in the form of a flattened mass. The shape, sizes and forms are changed according to the convenience of the practitioner.

Preparation of *Humao* as a starter by *Dimasa* tribes, Assam (Das and Deka, 2012)

Dimasa tribe of Assam prepared rice beer popularly known as *Judima*. The starter culture needed for Judima preparation is *humao.* For preparing *humao,* brown rice is soaked in water for 10-12 hours at room temperature. It is then crushed with the barks of *Glycyrrhiza glabra* L. The mixture is then made into paste by adding water and flat cakes are prepared from this mixture and sundried (Chakrabarty *et al.,* 2009). For preparing *judima,* rice is first cleaned and washed. This is then cooked and dewatered. After cooling it is mixed properly with *humao* in appropriate quantity. This mixture is then spread on a banana leaf for overnight and then transferred to an earthen pot and made partially air tight. Fermentation is allowed to take place at ambient temperature for 3-4 days during summer and 6-7 days during summer. The resultant juices are known as *judima* (Chakrabarty *et al.,* 2009). In similar manner the North East tribes used *Acacia pennata* bark, are cut into small pieces and dried in the sun. Rice is soaked in water until it is softened. It is then grinded in a wooden or metallic mortal pestle called *rimin* along with the barks of *Acacia pennata*. A little water is added in order to make a paste. They are then made into cakes of appropriate sizes and allowed to dry for a period of one week. They can be stored for many months (Das *et al.,* 2012).

Preparation of *Siiyeh/ Siye/Opop/Ipof/Ipoh* by tribes of Arunachal Pradesh (Das and Deka, 2012; Das *et al.*, 2012)

Apong and *Ennog/ Sai Modis* are alcoholic rice beverage prepared by the tribes of Arunachal Pradesh and the Mising tribes of Assam (Tiwari and Mahanta, 2007; Das and Deka, 2012). Both bears very important place in the tradition of the people of their region. The starter culture used for their preparation is called Siiyeh/ Siye/Opop/Ipof/*Ipoh* which contains the yeast to carry out the fermentation. For preparation of *ipoh* rice is first dried and grinded into fine powder. This is then mixed

with powder of seeds and barks of the locally available plants *Veronia cinerea* Less and *Clerodendron viscosum* Vent. This mixture is taken into a vessel called *Dekchi* and made into a paste using water of previously prepared *apong*. This paste is poured and spread on bamboo mats and made into disc shaped small cakes. They are then carefully dried over the fireplace or left in a cool place for 3 to 4 days. After drying they can be stored for up to a year (Tiwari and Mahanta, 2007). Similarly for preparation of Ennog/Sai Mod (black beer) rice is first boiled and spread on a bamboo mat to cool. Simultaneously, paddy husk is filled into a large tin sheet or drum and allowed to burn slowly and evenly till they become black. The burnt husk, while still hot is mixed with the boiled rice and allowed to cool. After cooling, the mixture is again mixed with crumbled *ipoh* cakes and transferred to bamboo basket for fermentation.

Preparation of *Khekhrii* by the *Mao* tribes of Nagaland (Mao, 1998)

Khekhrii is a unique starter culture prepared from fermented germinated rice in Nagaland by Mao tribes (Mao 1998). The ethnic alcoholic drink prepared using Khekhrii is called *zutho* or *zhuchu*. Water used for making *khekhrii* is traditionally brought in a gourd (*Lagenaria siceraria* [Molina] Standley) (family Cucurbitaceae) shell from a spring (Mao, 1998). Mao tribes believe that if water is brought in any other jar it may spoil the starter culture. Rice is collected and cleaned and put into an earthen jar that is filled with water brought in the gourd shell. Two pieces of charcoal and two fresh twigs of *Elscholtzia blanda* Benth. are also put into the jar. *Khekhrii* makers believe that the addition of charcoal pieces and *Elscholtzia blanda* are important, acting as antimicrobial regulators to keep fermenting rice from contamination. The mouth of the jar is closed tightly with fresh leaves of *Justicia adhatoda* Nees. and the jar is kept in a warm place for 7-14 days, depending on the room temperature. In general, it takes about a week in summer and two weeks in winter. At the end of fermentation, a typical flavor comes out when the mouth of the jar is opened. After fermentation, the contents of the jar are poured into a sieve and the water is discarded, and the fermented paddy is kept inside the basket. The basket is opened after germination of the paddy has taken place. The germinated paddy is then dried in the sun and stored in a dry container for use as a starter, called *khekhrii* which is pounded into powder and used in the preparation of *zutho* or *zhuchu*, a traditional alcoholic beverage of Nagaland.

Preparation of *Mod pitha* (natural starter) by *Deori* tribe of Assam (Deori *et al.*, 2007)

Saol (rice grains), plant species (Table 1), *Kula* (a round bamboo utensil), *Saloni* (round bamboo utensil or sieving), *Dheki* (wooden grinder), *Dhua sang* (a rectangular frame made of bamboo), *Soriya* (aluminium utensil) and *Kher* (straw) are required for the preparation of *Mod pitha* (Figs. 1-3). A handful each of cleaned leaves, fronds, barks, roots and bulb of the plant parts are put in a *Saloni* and kept for a day for sun drying. 3-5 kg of *Saol* is soaked in water for about 2 hrs, mixed with the dried plant materials and grounded in a *Dheki.* The grounded powder is taken out, sieved in a *Saloni* and the

coarse part is returned to the *Dheki* for grinding. The process is continued until a fine powder is obtained. 2-3 old *Mod pitha* are added to the mixture while grinding, which acts as an inoculant. Grounded powder is put into a *Soriya*, water is added to make a sticky paste and small round cakes (2-3 cm in diameter and *ca*.1 cm in thickness) are prepared. Cakes are then kept on clean, dry paddy straws spread on a *Kula* (a round bamboo utensil) and again covered with straws. *Kula* is then kept on a *Dhua sang* tied about 1 m above the fireplace in the kitchen for drying. This procedure of baking continues for a couple of weeks until the *Mod pitha* becomes hard. *Pitha* is then ready for use in *Sujen* brewing. Unused *Mod Pihta* is stored in *Tekele* (small earthen pot), mouth of which is covered with a bunch of straws. It can be stored for 2-3 months and can be used as and when required.

Preparation of *Marcha* by the tribals of Darjeling and Sikkim (Tamang, 2010)
Marcha are dry round to flattened, creamy, white to dusty white, solid ball which is used as an amylolytic starter to produce ethinic alcoholic beverage in the Himalaya Regions (Darjelling hills, Sikkim) of India, Nepal, Bhutan and Tibet in China (Tamang, 2010). It has various vernacular names among the Himalayan tribes viz. Phab by Tibetians and Bhutia; khesung by Limboo; Bharama by Tamang; Bopkha or Khabed by Rai, Buth/Thanbum by Lepcha; Poo by Drukpa, Manapu and Mana by Newar (Tamang, 2010).

For *marcha* preparation, glutinous rice (*Oryza sativa*) is soaked in water for 8–10 h at ambient temperature and unheated soaked rice is crushed in a foot-driven, heavy wooden mortar and pestle. In 1 kg of ground rice, ingredients added include roots of *guliyo jara* or *chitu* (*Plumbago zeylanica*), 2.5 g; leaves of *bheemsen paate* (*Buddleja asiatica*), 1.2 g; flowers of *sengreknna* (*Vernonia cinerea*), 1.2 g; ginger, 5.0 g; red dry chilli, 1.2 g; and previously prepared *marcha* as mother culture, 10.0 g. The mixture is then made into a paste by adding water and kneaded into flat cakes of varying sizes and shapes. These are then placed individually on the ceiling–floor, above the kitchen, made up of bamboo strips inlaid with fresh fronds of ferns (*Glaphylopteriolopsis erubescens*), covered with dry ferns and jute bags and are left to ferment for 1–3 days, depending upon the temperature. Completion of fermentation is indicated by a distinct alcoholic and ester aroma and puffy/swollen appearance of the *marcha* (Tamang, 2010).

Preparation of *Perok Kushi* by the Deoris tribals of Assam (Das *et al.*, 2012)
The indigenous rice beer of the *Deoris* is known as *Sujen*, prepared using starter material *perok kushi*. The plant materials used for preparing *perok kushi* are leaves of *Jasminum sambac*, *Cinnamomum byolghata*, *Zanthoxylum hamiltonianum*, *Lygodium flexuosum*, *Acanthus leucostychys*, *Cyclosorus exlensa*, *Alstonia scholaris* and roots of *Alpinia malaccensis* and the stem and rhizome of *Costus speciosus*. All these are washed and cut into small pieces, dried and grinded using a specialized wooden grinder called as *dheki*. The

mixture is then soaked in water in a vessel until the water becomes coloured. The whole mixture is added to grinded rice in a vessel in order to make dough. Round balls of about 4 cm diameter is made out of this and dried either in the sunlight or over the fire hearth by placing in a bamboo mat called as *aaphey*. After getting dried they are placed in a bamboo container called as *kula* the inside of which is laid with *kher* (paddy straw). Its mouth is again covered with *kher* and is kept over the hearth for storage. They can be kept in this manner for many months and can be used as and when required.

Preparation of starter (*Ranu dabai*) by the tribals of West Bengal (Ghosh and Das, 2004)

Ranu dabai is the starter used for preparation of Jhara/Harhia similar to Hnadia preparation by the tribes of West Bengal. *Ranu dabai* are the mixture of roots, barks, rhizomes, leaves of about 10-12 plant species (Table-1) and binded with the rice flour. 10 Kg of rice grains are taken on a flat traditional utensil generally made of sliced bamboo ('Soop') washed properly. Clean water is poured in it, stirred and decanted. The decanted wash-water is preserved in a bucket for future use. The most common plants viz. tuberous roots of *Coccinia grandis* (500 gm); leaves of *Clerodendrum viscosum* (300 gm); whole plant of *Vernonia cinerea (*350 gm) and leaves of *Plumbago zeylanica* (250 gm) are taken and chopped and ground properly on Soop. All rest plant (50-100 gm only) depending on the availability are taken and added for preparation which will improve the quality of the starter. In some cases 300 gm of *Rauvolfia serpentina* roots can replaces *Coccinia grandis* roots. Rice grains are then put in the pit of wooden husking machine (Dhiki) and where partially powdered a few (3–4 large tablets for 10 kg of rice) old *Ranu Dabai* are added. After some time, the plant paste is also added to it and allowed to mix properly. When the rice grains are properly powdered and mixed with plant paste, it is then taken out on a sieve (Chakni) and the coarse part is returned to the wooden husking machine. After completing sieving, woody and fibrous materials are rejected. The powdered material (Gunda) is now taken in a large vessel (Dikchi) and made into paste using the previously stored rice wash water. The paste becomes slightly greenish-white and emits the smell of mixed herbage. Clean gunny bags are then spread on the floor under shade or inside the rooms. Different size such as small, medium and large (standard size is 4.5-7 cm in diameter), are prepared by pressing with hand and arranged in rows on the gunny bags, where these are kept for 40-60 minutes. Tablets loose some amount of water and become little tough. All the materials are taken in a large basket (Dhakiya) made of sliced bamboo. Clean and dry straw is spread on the bottom of the basket and some tablets are kept on it. These are then covered with straw and another layer of tablets is kept on it. The entire set is covered with polythene sheet and/or gunny bags and stored in a dark and warm place. The incubation period varies from 2-3 days in warm season and 4–6 days in winter. The inside temperature rises considerably (fever) and the set starts emitting pungent Harhia-like smell. During this, a layer of cottony mycelia develops on the tablets. The fungal mycelia produce a mat of

black sporangia in damp weather or if stored for a slightly longer period. The tablets are taken out of the basket and are kept in single layer on large sized circular flat bamboo basket called 'Dagra' and get dried under the sun for 7–8 days. Now, the *Ranu Dabai* is ready for storing and for use.

Preparation of fermentation cake (*Ranu goti/ Ranu tablets*) by the tribals of Central India (Kumar and Rao, 2007)

In the preparation of *Handia* and *Mahua*, *Ranu* tablets play an important role, act as yeast starter or fermentor, and help in fermentation of both beverages. *Ranu* tablets or *Ranu goti* are the mixture of roots, barks, rhizomes, leaves of about 20-25 plant species (Table-1) and binded with the rice flour. The preparation is almost same with the preparation of Ranu dabai with only difference in plant species used and their ratio. For preparation of tablets, rice is soaked in water, pounded, and kept in shady place for drying. The plant species used in preparation of *Ranu goti* are collected mostly from forests in wild condition. The roots, leaves, bark, seeds of the plants are sun dried and pounded, powdered and dried in sun. The powder is mixed with flour thoroughly in the ratio of 1:2, and rolled in small pieces in the form of small cakes. These tablets are kept in closed room for drying. After drying, these *Ranu* tablets or *Ranu goti* are used for preparing local beverages *Handia*.

Preparation of *Thiat* by the tribes of Mahalaya (Samati and Begum, 2007)

Leaves of *Amomum aromaticum* Roxb. are cleaned, sun dried and ground into powder in a mortar made of hard wood of *Schima wallichii* (DC.) Korth (Thlong) by a pestle made of hard wood of *Docynia indica* (Wall.) Decne (*Sura*i). Then, 1-2 kg of *Oryza sativa* L. soaked in water is also ground in a mortar made of hard wood of *Schima wallichii* by a pestle made of hard wood of *Docynia indica* (Wall.) Decne (*Sura*i). The process is continued until a fine powder is obtained. *Thiat* (natural yeast) cakes are made from the ground rice powder mixed with *Amomum aromaticum* Roxb (Khaw-iang/Haw-iang) leaves powder, which are put in a cone-shaped basket (Khrie) and spring water (Um-pohliew) is added to make a sticky paste and small round cakes are prepared with standard size of 4-5 cm in diameter and 0.8-1cm in thickness. These cakes are then kept in a round basket (Malieng) and covered by leaves of banana. A round basket (Malieng) is hanged on a bamboo made a rectangular frame, which is exposed to sunlight or tied about 1.20-1.50m above the fireplace/hearth for drying until the cakes get harden and then are used for rice brewing as natural yeast.

Preparation of *Vekur pitha* by the Ahoms tribes of Assam (Saikia *et al.*, 2007)

Vekur Pitha is the starter prepared by the *Ahoms* tribes of Assam for preparation of rice beer *xaj-pani* (Saikia *et al.,* 2007). For preparing *vekur pitha,* a mixture of plant leaves viz. *Lygodium flaxuosum* Linn., *Leucas aspera* Spreng, *Cissampelos pereira*, *Scoparia dulsis* Linn., *Cinamommum glanduliferum* Meissn. and *Piper betle* Linn. are dried in the sunlight for 1-2

days. These are then grounded to powder and mixed with powdered rice in a vessel with some amount of water. To this powder of previously prepared *pitha* called *ghai pitha* is added which serves as a source of yeast. The mixture is then made into disc shaped cakes and wrapped with banana leaves (*Musa paradisiacal* Linn.) and kept in air locked condition above the fire heart for 4 to 5 days. After getting dried, the cakes are known as *vekur pitha* which serves as the source of *Saccharomyces cerevisae* and can be stored for future use.

MATERIALS AND METHODS

Survey and documentation of preparation of *Handia*: A survey was conducted in 10 selected localities (Palbani, Takatpur, Roxy Road, Chhaupadia, Baghra Road, Tulasichoura, Darogadahi, Station Bazar, Hospital Road and Baripada Hat) of Baripada city in the district of Mayurbhanj where preparation and large scale selling of *handia* is a normal practice. Information was collected on methods of preparation, raw materials used and frequency and timing of consumption through informal personal interview (N = 5 at each sampling point) among various tribal communities. Accuracy of the information was ensured through cross verification.

Survey and documentation of plants in Bakhar preparation: Survey was conducted in different villages of Mayurbhanj district of Odisha and gathered information through questionnaires and personal interaction with native tribal people regarding the use of plants and plant parts in the preparation of starter culture and its detail method of preparation is recorded. As most of the tribals obtain the principal constituents in form of the powder plant parts from the market, so a survey was conducted by showing the plant parts along with their dried form. Accuracy of the information was ensured through cross verification. The plant specimens used in Bakhar preparation were collected, identified and deposited as voucher specimen in Department of Botany, North Orissa University. Medicinal uses of the same plants were obtained by interviewing (once only) traditional healers of 24 villages of the district (Figure-2). All of them were males with an average age of 46 years. Prior informed consent was not taken from the informants as most of them were professionals and reputed in their respective areas and prescribed plant preparations for different ailments.

Figure-2: MAP of Mayurbhanj district with sampling site (*)

Collection and processing of plants: Bark, flower, leaves, roots, rhizome and young shoot of plants have separately been collected during field trips to different places of Mayurbhanj. The roots are dug out from the soil and the adhering soils were removed by shaking and washing. The leaves were plucked from the trees, washed properly and infected leaves were discarded. After collection, the healthy leaves were dried at room temperature to maintain their green color and volatile oils, if present. The material is completely shed dried so long it does not allow for the growth of any type of fungi, molds, bacteria and other microorganisms. The dried bark, flower, leaves, roots and rhizome are powdered separately by using mortar and pestle.

Extraction of plants: Hundred grams of each powdered samples were dissolved in 200ml of ethanol separately in wide mouth bottle. The suspension was then filtered (Whatman No. 40) separately and utilized for studying antimicrobial properties and phytochemicals. Ethanol extract was dried in rotary evaporator (Sonax, India) at 40 ˚C and store in refrigerator for further study.

Phytochemical Analysis:
Qualitative phytochemical analysis was carried out using methods described by Trease and Evans (1989). Each extract was screened for presence of alkaloids (using Mayer's, Wagner's, Hager's and Dragend dorff's reagents); flavonoids (NaCl and HCl); carbohydrates (using Molisch's reagent); glycosides (using Keller Killiani and Borntrager's reagents); protein and amino acids (using Biuret, Xanthoproteic, Ninhydrin and Millon's reagent); tannin and phenolic compounds ($FeCl_3$ and Gelatin); triterpenoids (thionyl chloride solution); steroid and sterols (using Liebermann Burchard and Salkowski's reagents), fat and fixed oils with alcoholic KOH reagents.

Antimicrobial activity
Enteric pathogens viz. enteropathogenic and enterotoxigenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*, *S. sonnei*, *Staphylococcus aureus* and *Vibrio cholerae* were used in the present study as discussed earlier by Panda *et al.,* (2010). The antibiogram was carried out by adopting disc diffusion method using several antibiotics. The result of the antibiogram was published in our earlier report (Panda *et al.,* 2010). The agar cup method and MIC were used to study the antibacterial activity. Broth microdilution technique adopted using 96-well microtiter plate and tetrazolium salt, 2,3,5-triphenyltetrazolium chloride (TTC), was carried out to determine the MIC following the method as described by Eloff, (1998). Selected extracts were serially diluted in the 96-well plate with an overnight culture of microorganisms (0.5 McFarland) grown at 37°C to obtain the final concentration of extracts ranging from 78 to 2500 µg/ml. The microplate was sealed and incubated at 37°C and observed for the growth of the microorganism. 10µl of the broth from each well of 96 microtiter plate (≤MIC) and control wells were taken aseptically and plated on one day old MH agar

plate as a point inoculum and allowed to dry for 10 min. under the laminar air hood. These plates were then sealed and incubated at 37°C for 24 hours and observed for growth of the bacteria. Absence of growth of the bacteria showed the MBC result of the respective bacteria.

RESULTS

Handia preparation includes two distinct phases: preparation of *ranu* or *bakhar* tablets and making of *handia*.

Ethnomedicinal uses, Preparation of *Bakhar* or *Ranu* tablets

Ranu or *bakhar* tablets act as starter for fermentation. *Ranu* tablets are mixtures of various plant parts (50%) and powdered un-boiled rice (50%). The plant species and parts thereof used for the purpose along with local names, family, parts used are listed in Table-2 along with its medicinal properties throughout India. Survey was carried out and plants are collected after documenting its ethnomedicinal uses are listed in Table-3 and Figure-3. Some species viz. *Asparagus racemosus* (Willd.), *Cissampelos pareira* L. *var. hirsuta* (DC) Forman, *Clerodendrum serratum* (L.) Moon, *Coccinia grandis* (L.) Voigt, *Holarrhena antidysenterica* Wall ex. A. DC., *Woodfordia fruticosa* (L.) Kurz. and Benth. are commonly used by the tribal of all localities while plants such as *Madhuca longifolia* (Koenig), *Smilax macrophylla* (Roxb.), *Rauwolfia serpentina* (L.), *Elephantopus scaber* L., *Gardenia gummifera* L.f. and *Dioscorea* sp. are rarely use. Depending on the season and availability in a particular locality, plant parts of one or more species are used. The accurate ratio of different plants used for *ranu* preparation could not be ascertained as the informants were reluctant to disclose the same. However, *C. pareira* forms the major part in most of the preparations (70%) followed by other plants in combination (1-30%). *R. serpentina* and *G. gummifera* are used in very small proportion. According to one informant (Sama Singh, Male, Age-62) the ratio of the plant (root) is 6:2:1:1 (*C. pareira*: *W. fruticosa: A. racemosus*: *H. antidysenterica*). Preferred parts and plants varied at different places. Dried root, stem and other parts used for the purpose, both as such and powdered, are abundantly and openly sold in the local markets (Figure-4. a & b). Powdered plant ingredients are mixed with equal amount of rice (*Oryza sativa* L.) powder. A suitable amount of water is added to make dough. *Ranu* is prepared in the form of rounded tablets and spread over straw beds in layer over layer with a final thin layer of straw cover. After 3 days, the *ranu* tablets are picked up from straw beds and dried under sun for about 2 days and stored for use in fermentation of rice beverages (Figure- 3. e). These tablets are not only used for fermenting rice beverage but also used for treatment of various ailments.

Figure-3: Plant parts used for preparation of Bakhar

a. *Asparagus racemosus* root
b. *Cissampelos pareira* root
c. *Clerodendrum serratum* root
d. *Coccinia grandis* rhizome
e. *Dioscorea* sp. tuber
f. *Elephantopus scaber* root
g. *Holarrhena antidysenterica* bark
h. *Madhuca longifolia* flower
i. *Woodfordia fruticosa* root

Table-2: Phytotherapeutic uses of plants that are used in preparation of *ranu* tablets

Sl. No.	Name of the plant	Local name	Family	Parts used	Medicinal uses	References
1.	*Asparagus racemosus* Willd.	Gaisiro	Liliaceae	Root	Nutritive tonic and demulcent, cures fever, sexual debility, leprosy, bronchitis and cough.	Thatoi *et al.*, 2008; Wani *et al.*, 2011
2.	*Cissampelos pareira var. hirsuta* (Buch.-Ham. ex DC.) Forman	Andiakidula	Menisper-maceae	Root, Leaf	Leaves used for cough, urinary troubles, diarrhea, inflammation and colic pain.	Rout & Panda, 2010; Thatoi *et al.*, 2008
3.	*Clerodendrum serratum* (L.) Moon	Samarkand	Verbenaceae	Root, Leaf	Roots used in fever, snakebite, asthma and cough.	Kirtikar *et al.*, 2005
4.	*Coccinia grandis* (L.) Voigt	Banokunduri	Cucurbitaceae	Root tuber	Ear pain, jaundice, blood dysentery and diabetes.	Panda *et al.*, 2011; Tamilselva *et al.*, 2011
5.	*Dioscorea* sp.	Sanga	Dioscoreaceae	Root tuber	Not known.	
6.	*Dipteracanthus suffruticosus* (Roxb.) Voigt	Chaulia	Acanthaceae	Root	Renal problems.	Nayar *et al.*, 1956
7.	*Elephantopus scaber* L.	Tatmuli	Asteraceae	Root	Diarrhea, dysentery, colic, vomiting, headache and tooth ache.	Panda *et al.*, 2011

8.	*Gardenia gummifera* L.f.	Bhurudu	Rubiaceae	Young shoot	Used as a digestive tonic and antiseptic.	Kirtikar *et al.*, 2006
9.	*Holarrhena pubescens* (Buch.-Ham.) Wall. ex. G. Don	Kuruchi	Apocyanaceae	Bark, Seed	Bark is antihelminthic, antipyretic and used in dysentery. Seeds are astringent, used in cough, cold, fever, scabies, leprosy and malaria.	Kirtikar *et al.*, 2006; Panda *et al.*, 2011; Thatoi *et al.*, 2008
10.	*Homalium nepalense* (Wall.) Benth.	Danmari	Flacourtiaceae	Bark	Stomach disorder.	Nayar *et al.*, 1956
11.	*Lygodium flexuosum* (L.) Sw.	Mahajal	Lygodiaceae	Root	Fresh roots used as expectorants and healing wounds.	Panda *et al.*, 2011
12.	*Madhuca longifolia* (Koenig) MacBride *var. latifolia* Roxb.	Matkam	Sapotaceae	Seed, Leaf, Bark	Seed oil used in rheumatism. Leaf and bark used for diabetes.	Kirtikar *et al.*, 2006 Nayar *et al.*, 1956
13.	*Ochna obtusata* DC. *var. obtusata*	Otchampa	Ochnaceae	Root	Used as antidote for snake bite, menstrual complaints and asthma.	Kirtikar *et al.*, 2005[17]
14.	*Orthosiphon rubicundus* (D.Don) Benth.	Chandua	Lamiaceae	Root tuber	Used to cure stomach disorder.	Nayar *et al.*, 1956
15.	*Polygala crotalarioides*	Lilkathi	Polygalaceae	Bark	Cold decoction of bark for cough. Used	Nayar *et al.*, 1956

	Buch.-Ham. ex. DC				as antidote against snake bite.	Kirtikar *et al.*, 2005
16.	*Phoenix acaulis* Roxb. ex. Buch.-Ham.	Khajuri	Arecaceae	Root	Used as laxative.	Nayar *et al.*, 1956
17.	*Rauwolfia serpentina* (L.) Benth.	Patal Garuda	Apocynaceae	Root	Used as antidote for snake bite, malaria.	Nayar *et al.*, 1956 Rout & Panda, 2010
18.	*Smilax macrophylla* Roxb.	Ramadantani	Smilaceae	Root, Stem	Roots used for urinary complaints and dysentery. Stem used in tooth ache.	Panda *et al.*, 2011
19.	*Woodfordia fruticosa* (L.) Kurz	Icheba	Lythraceae	Flower	Dried flowers used as astringent, leprosy, burning sensation and liver disorders.	Panda *et al.*, 2011; Thatoi *et al.*, 2008
20.	*Xantolis tomentosa* (Roxb.) Raf.	Ghurmur	Sapotaceae	Fruit	Used as an antiseptic and digestive tonic.	Rout & Panda, 2010

A paste of *ranu* tablets with saliva is applied on mumps by the tribes before sleeping to get relief. Santal tribals also disinfect silk worm (tasar) eggs during indigenous rearing. From the study conducted in the various villages, it is evident that all tribes use some plants for starter preparation; however the type of plant varies from tribe to tribe and village to village. The tribal peoples of Sukruli and Karanjia block use the same plant viz. *C. pareira, C. serratum, C. grandis*, *Dioscorea* sp. for starter preparation. Similarly tribes of Bijatola and Rairangpur use the

Figure-4: Preparation of Bakhar

a. *Clerodendrum serratum* root powder
b. *Cissampelos pareira* root powder
c. *Asparagus racemosus* root powder
d. *Holarrhena antidysenterica* bark powder
e. Drying of Bakhar in mats
f. Bakhar in large quantity for sale at Baripada hata
g. Bakhar prepared by a tribal (male) at Gorumahisiani, Mayurbhanj

same plant in addition to *A. racemosus* instead of *Dioscorea* sp. However, the tribes of Samakhunta use different plants *A. racemosus*, *C. pareira*, *H. antidysenterica, M. longifolia, S. macrophylla* and *W. fruticosa* for the same purpose. This is an age old practice in these communities which is followed generation after generation.

Preparation of *Handia*

Mechanically de-husked rice is soaked and boiled in water. The cooked rice is dried on a bamboo mat under sun (Fig. 1d). After drying, the rice is mixed with required amount of powdered *ranu* tablets (approximately 10 tablet per kg rice), kept in a large earthen pot or *handi* (hence the name of the product) followed by addition of required amount of water.

Table-3: Ethnomedicinal uses of plants used for preparation of Bakhar

Sl. No.	Name of the plant	Ethno-medicinal uses
1.	*Asparagus racemosus* Willd.	Root paste in water is administered (10 g) two times a day in gonorrhea and jaundice. Root is taken as tonic.
2.	*Cissampelos pareira* L. *var. hirsuta* (DC) Forman	Filtered root juice is taken with water to cure colic.
3.	*Clerodendrum serratum* (L.) Moon	Paste of leaves applied locally for treatment of septicemia, worms and foot diseases
4.	*Coccinia grandis* (L.) Voigt	Fresh leaves along with leaves of *Kalanchoe pinnata* and sugar are ground with water and taken twice a day for four to five days to cure jaundice.
5.	*Dioscorea* sp.	Tuberculous rhizome are cooked and eaten as famine food.
6.	*Elephantopus scaber* L.	The entire plant is cooked with rice and eaten to cure migraine. An entire root is tied over forehead to get relief from headache.
7.	*Gardenia gummifera* L.f.	The leaves are used in stomach pain and stems against teeth pain.
8.	*Holarrhena antidysenterica* Wall ex. A. DC.	Stem bark infusion with honey (3:1) is taken once a day in empty stomach for cure of dysentery. Bark of the plant and black pepper are powered together and orally taken against malarial fever.
9.	*Madhuca longifolia* Koenig	Flowers are boiled in water with a pinch of salt and the decoction is given with honey, thrice a day for seven days for cure of piles and fistula.
10.	*Rauwolfia serpentina* (L.) Benth.	Juice extracted from leaves mixed with the juice of *Andrographis paniculata* and *Azadirachta indica* and

		given it with honey to drink for seven day continuously to cure malaria.
11.	*Smilax macrophylla* Roxb.	Roots are boiled in water, is given orally with honey to cure gastric problems.
12.	*Woodfordia fruticosa* (L.) Kurz	Juice is good for treating dysentery. Leaves are used for edible purpose.

The mixture is kept untouched for 3-4 days for fermentation. After proper fermentation a white supernatant was present at the upper layer containing 8-10% alcohol called *rashi*, which fetches higher price. After 2-3 days the fermented liquid is allowed to trickle down through a bamboo sieve, collected in earthen pots and is ready for consumption (Fig. 1e &f). The taste of *handia* depends on the plants used for *ranu/bakhar* preparation. About 8-10 *bakhar* tablets are used for 1 kg of rice, which together produce about 10 L of *handia*. The quality gets lowered on dilution. On an average, some 30% of families prepare *handia* for their own consumption. Per capita consumption amounts to be about 1 L/day. Consumption is much higher during summer and hence, it is essentially a summer drink (Figs. 1g & h). It keeps the stomach cool, protects from extreme heat and is also intoxicating. *Handia* preparation and selling is a secondary source of livelihood for tribals and some accept it as a primary occupation. It is used in all social, cultural and religious purposes and no social occasion is considered complete without it. The Santals believe it to possess medicinal value, i.e. use it in the cure of jaundice, colic disorders and dysentery.

Screening of phytochemicals and antimicrobial activity of plants parts used in starter

The qualitative phytochemical analysis of ethanol extracts indicated the presence of alkaloid, flavonoid, carbohydrate, protein and amino acid, tannin and phenolic compounds and saponin in most of the plants (Table-3). However, glycoside, steroid & sterols, gum & mucilages, oil & fats and triterpenoids were found in less number of test plants. Alkaloid, carbohydrates, and saponin are universally present in all test plant extracts. Preliminary screening of antimicrobial activity was evaluated by using agar cup method against eight human pathogenic bacteria are given in Table-4. The zone of inhibition was found maximum against *S. aureus* followed by *S. sonnei* and *S. flexneri*. Organism such as *S. typhimurium* and *V. cholerae* show moderate zone of inhibition while enteropathogenic and enterotoxigenic *E. coli* and *P. aeruginosa* show least zone of

Table-4: Phytochemical screening of plant parts used for preparation of Bakhar

Name of the phytochemicals	Qualitative test	Ar	Cp	Cs	Cg	Ds	Es	Gg	Ha	Ml	Rs	Sm	Wf
Alkaloid	MR	-	+	+	+	+	+	+	+	-	+	+	+
	DD	+	+	-	+	-	+	-	+	-	-	+	+
	HR	-	+	+	+	+	+	-	+	-	+	+	+
	WR	-	-	-	+	-	-	+	-	-	-	-	+
Carbohydrates	MT	+	+	+	+	-	+	+	+	+	+	+	+
	FT	+	+	+	+	+	+	+	+	+	+	+	+
	BT	+	+	-	+	+	+	+	+	+	-	+	+
Tannin and Phenolic compounds	WFC	-	+	+	+	-	-	+	+	+	+	-	+
	WLA	-	-	+	-	-	-	-	+	-	+	-	-
	WGS	-	-	-	-	-	-	-	-	-	-	-	-
Glycoside	KKT	+	-	+	+	-	-	-	+	+	-	-	+
	LT	+	-	-	-	-	-	-	-	-	-	-	-
	BRT	-	-	-	-	-	-	-	-	-	-	-	-
Proteins and amino acids	BIT	-	+	+	+	+	+	+	+	+	-	-	+
	NT	-	+	+	+	+	+	+	+	+	+	-	+
	XT	-	+	+	+	-	+	+	+	+	+	-	+
	MLT	-	+	+	+	-	-	-	-	-	-	-	+
Flavonoids	WN	-	+	+	+	-	+	-	+	-	+	+	+
	WS	-	+	+	+	-	+	-	+	-	+	+	+
Saponins	HCT	+	+	+	-	-	+	+	+	+	+	+	+
	FT	+	+	+	+	+	+	+	+	+	+	+	+
Steroids and sterol	ST	-	-	+	+	-	-	-	-	-	-	-	+
	LBT	-	-	+	+	-	-	-	-	-	-	-	+
Triterpenoids	TCT	-	-	+	+	-	-	-	+	-	-	-	-
Oil and fats	WFC	+	-	+	-	-	-	-	+	-	-	-	-

Ar- *Asparagus racemosus*; **Cp-** *Cissampelos pareira*; **Cs-** *Clerodendrum serratum*; **Cg-** *Coccinia grandis*; **Ds-** *Dioscorea* sp.; **Es-** *Elephantopus scaber*; **Gg-** *Gardenia gummifera*; **Ha-** *Holarrhena antidysenterica*; **Ml-** *Madhuca longifolia*; **Rs-** *Rauwolfia serpentina*; **Sm-** *Smilax macrophylla*; **Wf-** *Woodfordia fruticosa*; MR- Mayer's reagent; DD- Dragend droff's test; HR- Hager's reagent; WR- Wagner's reagent; MT- Molisch's test; FT- Fehling's test; BT- Benedict's test; WFC-With Ferric chloride; WLA-With lead acetate; WGS-With gelatin solution; KLT-Keller-Killiani test; LT-Legal Test; BRT-Borntrager's test; BIT-Biuret test; NT-Ninhydrin test; XT-Xanthoproteic test; MLT-Millon's test; WN-With NaOH; WS-With H_2SO_4; HCT-Honeycomb test; FT-Foam test; ST-Salkowski's test; LBT-Liberman Burchard ; TCT-Thionylchloride test; WFP-With filter paper; (-) Absence; (+) Presence

inhibition. No test organisms were inhibited by *A. racemosus* (root), *C. pareira* (leaf), *Dioscorea* (tuber), *R. serpentina* (leaf) extract. So these plant extracts are not subjected to further study on MIC and MBC. The MIC results among all test bacteria are summarized (Table-5) and showed that extracts were able to prevent the growth of most of the test strains with selective activities. The growth inhibition of the test bacteria ranged from 125 µg/ml (w/v) to 1000 µg/ml (w/v) with the lowest MIC value against *S. aureus* (125, 156, 250 µg/ml) followed by *S. sonnei* (156, 250, 312, 500, 625 µg/ml). MBC test showed that in between 1000-2500 µg/ml (w/v) concentrations, most of test bacteria were killed.

DISCUSSION

Preparation and consumption of cereal based beverages is a common practice in many Mid-Asian, Middle East and African countries (Hesseltine, 1979). These are known by different names at different places: *sake* in Japan, Shaosingiju and *lao-chao* in China, Chongju and Takju in Korea, Brem bali, Tapuy and *Tape ketan* in Indonesia, *khao-mak* in Thailand, Tapai pulal in Malaysia and *jhara*, *daru*, *kali*, *pachwai*, *apong, bunkchung, chi, laopani, jumai, suze, morpo, jou, zu, mod*, *harhia* and *handia* in India (Lee, 2009).

Table-5: Screening of antibacterial activity of ethanol extracts of plants

Name of the plant	Parts tested	Zone of inhibition in mm (extract volume 40 µl from 20 mg/ml)							
		EP	ET	Pa	Sa	Sf	Ss	St	Vc
Asparagus racemosus	Root	-	-	-	-	-	-	-	-
Cissampelos pareira	Leaf	-	-	-	-	-	-	-	-
	Root	-	-	12	16	14	16	-	-
Clerodendrum serratum	Leaf	12	-	-	-	10	-	14	-
	Root*	15	12	12	14	17	16	15	14
Coccinia grandis	Rhizome*	-	-	-	14	15	12	14	12

Dioscorea sp.	Rhizome	-	-	-	-	-	-	-	-
Elephantopus scaber	Root	-	-	-	12	10	-	14	-
Gardenia gummifera	Young shoot	-	-	-	12	-	-	-	-
Holarrhena antidysenterica	Bark*	12	-	11	14	12	14	10	12
Madhuca longifolia	Flower*	-	-	-	16	14	12	10	-
Rauwolfia serpentina	Leaf	-	-	-	-	-	-	-	-
	Root	-	-	-	16	12	14	-	10
Smilax macrophylla	Root	-	12	-	14	-	12	-	-
Woodfordia fruticosa	Leaf	-	14	13	15	-	15	12	12
	Root*	13	18	12	18	12	14	16	14
Ciprofloxacin		15	20	17	24	18	26	24	22

Enteropathogenic *Escherichia coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), *Pseudomonas aeruginosa* (Pa), *Salmonella typhimurium* (St), *Shigella flexneri* (Sf), *S. sonnei* (Ss), *Staphylococcus aureus* (Sa) and *Vibrio cholerae* (Vc), C-Ciprofloxacin, *Stock concentration (25mg/ml)

Figure-5: Selling of Bakhar at different localities

a. A women selling bakhar, rice and plant powder at Station Bajara hata, Baripada
b. A seller selling bakhar and plant powder at Dhenkikote hata, Mayurbhanj
c. A seller selling bakhar at Kaptipada hata, Mayurbhanj

These products have many advantages like superior digestibility and nutritive value compared to their unfermented counterparts. The consumption of rice beer prepared from rice is a common practice among many tribal communities residing among the tribals of Odisha and many of them have been preparing it since time immemorial. Among the *Santal* tribes of Mayurbahnj, it is prepared in almost every third house. More or less all tribals are fond of drinks and consume during every ceremony, festivals, marriages, funeral feasts, and offer it to their, guests, gods and deities[7]. In marriages, the amount of *handia* to be given to the girl side and is decided well in advance.

Table-6: Results of screening of selected extracts for MIC and MBC by 96 well microtiter plate

Plant parts used	Plant extracts in µg/ml							
	Minimum Inhibitory Concentration in µg/ml				Minimum Bactericidal Concentration in µg/ml			
	Sa	Sf	Ss	St	Sa	Sf	Ss	St
CpR	125	500	250	1000	1000	>2000	1000	>2000
CsR	125	250	250	500	1000	1000	1000	2500
CgRh	156	312	625	312	1250	2500	1250	2500
EsR	125	250	250	250	1000	1000	1000	2000
HaB	156	156	156	312	1250	2500	1250	>2500
MlF	156	312	312	625	1250	2500	>2500	>2500
RsR	250	250	500	1000	1000	1000	>2000	>2000
SmR	250	1000	250	1000	1000	1000	1000	>2000
WfL	250	1000	250	250	2000	2000	2000	2000
WfR	156	156	312	625	1250	2500	1250	>2500

Sa- *Staphylococcus aureus*; **Sf-** *Shigella flexneri*; **Ss-** *S. sonnei*; **St-** *Salmonella typhimurium*; **Cpr-** *Cissampelos pareira* root**; Csr-** *Clerodendrum serratum* root**; CgRh-** *Coccinia grandis* rhizome**; Esr-** *Elephantopus scaber* root**; Hab-** *Holarrhena antidysenterica* bark**; MlF-** *Madhuca longifolia* flower**; RsR-** *Rauwolfia serpentina* root**; SmR-** *Smilax macrophylla* root**; WfL-** *Woodfordia fruticosa* flower; **WfR-** *Woodfordia fruticosa* root

The consumption of rice beverage emerged mainly due to the climatic conditions and discovering the use of surrounding natural resources (Roy *et al.,* 2004). There are also reports of rice beer being used as a drug from North east region of India (Singh and Singh, 2006). It works effective against insomnia, headache, body ache, inflammation of body parts, diarrhoea and urinary problems, expelling worms and as a treatment of cholera (Samati and Begum, 2007; Deka and Sarma, 2010). It cures jaundice, colic, and dysentery. It protects from sun stroke and maintains the motility and tone of the gastro-

intestinal system. It is taken as a light tranquillizer by *Maria* tribe of Baster (Sahu, 1996). It is also given to treat fever, dysentery, diarrhea and gynecological complaints (Usha, 1999). *Mahua* is given to treat dysentery by *Baiga, Gond*, and *Kol* tribes of Surguja district (Kumar and Rao, 2001). *Ranu* tablets are also used in treating cholera by *Gond* tribe of Surguja district (Kumar and Rao, 2001). The tribes of Mayurbahnj use *handia* in the cure of jaundice, colic disorders and dysentery (Panda *et al.,* 2014).

Plant ingredients are used in preparation of starter culture (*ranu* or *bakhar*) universally that are essential for fermentation of rice to prepare beverages. After surveying 12 districts of Odisha including Mayurbhanj (Dhal *et al.,* 2010) recorded the use of bark and root of six plant species in the preparation of *bakhar* by the tribals.

Singh, 1996 has reported the use of several plant species viz. mature leaves of *Allophylus cobbe* (L.) Blume**,** *Antidesma roxburghii* Wall. ex Tul. and tender leaves of *Artocarpus heterophyllus* (Lam.), in equal proportions together with little chilli, are used in the preparation of *Choarak*, a local wine of Tripura state, India. Tribal inhabitants of tea gardens in Terai region of West Bengal use 12 plants, specific parts for particular purpose, for preparation of rice beer *jhara* or *haria* (Ghosh and Das, 2004). According to Ghosh and Das, 2004, Sweetness of the liquor is developed by the use of tuberous roots of *Coccinia grandis* L. (Voigt), whole plant including fleshy roots of *Veronia cinerea* L. (Lessing) and leafy twigs of *Scoparia dulcis* L.. Likewise, young and soft leaves of *Clerodendrum viscosum* Ventinat, bark of *Oroxylum indicum* (L.) Benth. Ex. Kurz, root bark of *Rauvolfia serpentina* (L.) Benth. Ex. Kurz, and bark of *Wattakaka volubilis* (L.f.) Staph. Produce a bitter taste. Leafy branches of *Plumbago zeylanica* (L.) act as a process enhancer. Roots of *Stephania japonica* (Thumb.) Miers and *Stephania glabra* (Roxb.) Miers, are used for long storing. Roots of *Mussaenda roxburghii* (Hook.f.) and leaves of *Artocarpus heterophyllus* (Lam.), impart sweetness and yellowish tint to the liquor. The tribals of Central India use a number of roots, bark, rhizomes, leaves and seeds of some 21 plants for making *ranu* (Kumar and Rao, 2007). The tribals get the phytotherapeutic value of these plants through the drink. The use of specific plants and parts thereof is a tradition and passed through generations. However, due to the loss of biodiversity and fragmentation of habitat, all the plants are not available in the vicinity of a particular village.

Wani *et al.,* 2011 screened presence of phytochemicals in *A. racemosus* root from Himalaya region, India. According their findings, both alcoholic and aqueous extracts confirm presence of carbohydrate, glycoside, mucilage and saponin. In a study conducted by Mandal *et al.,* 2010 have shown antibacterial property of methanol extract of *A. racemosus* root against *Escherichia coli, Shigella dysentrae, S. sonnei, S. flexneri, Salmonella typhimurium, Pseudomonas putida* and *Staphylococcus aureus*. However, in the

present investigation antibacterial activity was not recorded against any test pathogens with the ethanolic extracts of *A. racemosus*.

Jhuma *et al*., 2012 studied phytoconstituents from methanolic extracts of *Cissampelos pareira* flower and result revealed presence of alkaloid, carbohydrate, flavonoid, protein, tannin and terpenoids. In the present study similar results were obtained for presence of phytochemicals in the ethanol extracts except triterpenoids. On the other hand, leaf extract don't show antibacterial activity against any strain while root extract show good inhibitory property against *S. aureus* and *S. sonnei*. This may be due to presence of certain additional phytochemicals in root comare to leaf.

Prasad *et al.,* 2012 reported presence of alkaloid, anthroquinoles, glycoside, phenol, saponin, tannin and terpenoids in *Clerodendrum serratum*. The same authors also evaluate the antibacterial activity of various extracts and result found that isoamyl alcohol had better antibacterial property against *Bacillus subtilis, S. aureus, S. typhi* and *Proteus* species. Similar type of results was also recorded by Vidya *et al.,* 2010 during their study on the same plant. Khatun *et al.,* 2012 studied the antibacterial activity of various extracts of *Coccinia grandis* and result found that methanol extracts showed antibacterial activity against *S. aureus, S. dysentrae, E. coli* and *S. typhi*. These authors also investigated presence of phytochemicals such as flavonoids, phenols, saponin, tannin and terpenoids. Similar study conducted by Umamaheswari and Chattargee, 2008 shown presence of phytoconstituents viz. alkaloid, glycoside, flavonoid, phenols, saponin and tannin. Presence of triterpenoids reported by Syed *et al.,* 2009 whereas proteins and aminoacid by Umamaheswari and Chattargee., 2009 while presence of both these phytochemicals in the ethanol extract right through the present study. Povendran et al., 2011 reported antibacterial activity of leaf extracts of *C. grandis* against *Heliobacter pylori*. Nevertheless, in the present study the rhizome extract exhibited very good antibacterial activity against *S. aureus, S. flexneri* and *S. typhimurium*.

Poli et al., 1992 evaluated pharmacological properties of *Elephantus scaber* and observed the presence of alkaloid, anthocyanin, chalcones, flavonoid, lactone and triterpenoids. In another study conducted by Kamalkannan et al., 2012 showed presence of alkaloid, carbohydrate, proteins and saponin while absence of volatile oil, gum, mucilage and steroid. The same authors studied the antibacterial property and conclude that the methanol extract of *Elephantus scaber* has potential antimicrobial activity against *Streptococcus pyogene* while no activity against *E. coil, P. aeruginosa, S. aureus and S. typhi.* In contrary to this work the study conducted by Kumar et al., 2004 and Avani and Neeta, 2005 reported promising antibacterial activity against pathogens such as *B. subtillis*, *E. coil, P. aeruginosa* and *S. aureus.* At this juncture, antibacterial activity was confirmed against *S. aureus, S. typhimurium* and *S. flexneri* whereas absence of activity against *E. coli, P. aeruginosa, S. sonnei* and *V. cholerae*. Tambekar and Khante[23]

evaluated antibacterial activity of *Gardenia gummifera* and result originated that this plant exhibit activity against *Enterococcus aerogenes, Klebesiella pneumonae, S. aureus* whereas no activity was evidenced against *E. coli, P. aeruginosa, S. typhi, Proteus vulgaris* and *S. typhimurium.* These authors also reported presence of phytochemicals viz. alkaloid, flavonoid, glycoside, steroid, tannin and phenolic compounds whilst absence of carbohydrate, protein and aminoacid. On the other hand, in the present study antibacterial activity was not observed by ethanol extract of *G. gummifera* except *S. aureus* (zone of inhibition 12 mm).

Ballal et al., 2011 studied antibacterial activity of *Holarrhena antidysenterica* against enteric pathogens and outcome confirmed antibacterial activity against EPEC, EIEC, *P. aeruginosa, Shigella boydii, S. flexneri, V. cholerae, S. aureus* and *S. typhimurium*. Study conducted by Preethi et al., 2010 validated antibacterial activity of *H. antidysenterica* against *B. subtillis*, *E. coil, S. aureus* and *S. typhimurium.* Chakraborty and Brantner, 1999 reported antibacterial activity of steroid alkaloid from stem bark of *Holarrhena pubescens against B. subtillis*, *P. aeruginosa, S. aureus, S. epidermidis, Streptococcus faecalis* and *S. typhimurium.* The present findings correlate with the study conducted by all these authors, relating to the antibacterial activity. Chakma, 2011 studied antimicrobial activity of *Madhuca longifolia* fruit and conclude that the plant act as a potential agent against *B. subtillis*, *E. coli, P. aeruginosa* and *S. aureus.* Later, Gopalkrishnan and Shimpi, 2012 carried out pharmacological studied on stem bark of *Madhuca longifolia* and result evaluated the presence of starch, protein, terpenoid, glycoside, saponin, tannin while absence of alkaloid, flavonoid and steroid. In the present investigation, antibacterial activity was recorded against *S. aureus* while *E. coli* and *P. aeruginosa* was not inhibited by *M. longifolia* extract. Nevertheless, the extract revealed presence of phytochemicals such as carbohydrate, protein, terpenoid, glycoside, saponin and tannin, and these finding are correlate with the study conducted by Gopalkrishnan and Shimpi, 2012.

Harisaranraj et al., 2009 examine phytochemicals such as alkaloid, flavonoid, phenolic compounds and tannin in the root extracts of *Rauwolfia serpentina.* Deshmukh et al., 2012 studied on antimicrobial activity of indole alkaloids from *R. serpentina* and conclude that root extracts illustrate better antimicrobial activity in compare to leaf extracts against *B. subtillis, E. coli, S. aureus* and *S. typhimurium*. The same authors at preliminary level investigated presence of phytochemicals viz. alkaloid, tannin, saponin, flavonoid and starch. Deshwal and Vig, 2012 studied antibacterial activity of *R. serpentina* and proved that ethanol extract showed higher zone of inhibition compare to norofloxacin against *S. aureus*. In the present experiment, ethanol extract of root exhibited antibacterial activity against *S. aureus, S. sonnei, S. flexneri* and *V. cholerae* while leaf extract do not inhibit any test strain. Furthermore, the phytochemical study correlate with the study carried out by Deshmukh et al., 2012.

Hooda et al., 2011 reported presence of phytochemicals such as carbohydrate, protein, saponin, flavonoid, alkaloid and tannin in the root extracts of *Smilax zeylanica*. The present study finds presence of phytochemicals such as carbohydrate, saponin, flavonoid and alkaloid. Nonetheless, antibacterial activity is the primary information reported on ethanol extract of this plant.

Parekh and Chanda, 2007 studied antibacterial activity of *W. fructicosa* flowers and result found that methanol extract exhibited potential activity against *B. cereus, S. aureus, S. epidermidis*, *P. vulgaris, P. seudoalcaligens* and *S. typhimurium*. Later, similar experiment carried out by Kumarswamy et al., 2008 showed promising antibacterial activity against *E. coli, K. pneumoniae*, *P. aeruginosa, P. mirabilis, S. typhi, S. boydii, S. flexneri, S. sonnei* and *S. aureus*. Chougale et al., 2009 also studied antibacterial activity of fraction of leaf extracts of *W. fructicosa* with promising antibacterial activity against *B. subtillis, E. coli, S. aureus* and *P. aeruginosa*. Further, Bhattarai and Bhuju, 2011 tested both leaf and flower extracts of *W. fructicosa* and concluded that methanol extracts have antibacterial activity against *B. cereus, E. coli, P. aeruginosa, P. mirabilis, S. dysentriae, S. typhimurium* and *S. aureus.* Finose and Devaki, 2011 investigated presence of phytochemicals viz. carbohydtate, aminoacid, glycoside, saponin, flavonoid, alkaloid and tannin in *W. fructicosa*. Later, Gyawali et al., 2012 establish presence of same phytoconstituents in addition with terpenoids.

The detail study and uses of these plants clearly indicate that the presence of these plant materials and their bactericidal activity are mainly responsible for protecting and preserving starter cultures since traditional system of fermentation normally operates in unhygienic condition which sometimes contaminates the system and cause toxication of drinks. But due to the presence of antimicrobial chemical principles of these plants or plant parts, they are able to continue such practice for generations without much decline in the characteristics of microorganisms involved in fermentation.

CONCLUSIONS

The study establishes the deep knowledge of tribal people in terms of selection of plants for preparation of *handia* and the processing technique. The native skill of rice beer preparation using unique starter culture technique is well recognized. Both the *Bakhar* tablets and *handia* are used in the treatment of several ailments. Probably, this is central theme in the preparation of rice beverages by the tribals across the world.

RECOMMENDATION

It is possible that the traditional knowledge of beverage preparation along with various parts of different plant species used will be a useful lead for phytochemists and pharmacologists for further study. Once the efficacy of these indigenous drinks is scientifically established, the popularization of these remedies can be recommended in

conventional healthcare systems for wider applications. Further work is required to fully characterize the predominant microorganisms and to establish their technological roles and contribution to product quality and safety.

ACKNOWLEDGEMENTS

The present research has been funded by the Department of Science and Technology, Government of Odisha (Grant No. ST-BIO-70/2010/ST). We wish to express our profound gratitude to Dr. A. K. Biswal (Department of Botany, North Orissa University) for identification of the plant samples. We are also grateful to the authorities of North Orissa University for providing necessary facilities to carry out this research. Finally, we wish to thank to all the informants and sellers; they allowed us to take photographs of plant materials. We also thank to Dr. Abhijeet Das for photographic documentation.

REFERENCES

Avani K and Neeta S (2005). A study of the antimicrobial activity of *Elephantopus scaber*. *Ind. J. Pharmacol.* 37(2): 126-128.

Ballal M, Srajan D, Bhat KK, Shivwaikar A and Shivananda PG (2011). Antibacterial activity of *Holarrhena antidysenterica* (Kuruchi). *Ind. J. Pharmacol.* 33: 392-393.

Bhattarai S and Bhuju DR (2011). Antimicrobial activity of useful parts of *Woodfordia fruticosa* (Linn.) Kurz. of Nepal. *Int. J. Pharma. Bio. Arch.* 2(2): 756-761.

Chakma CS (2011). Antimicrobial activity of the fruit seeds *Madhuca longifolia* (Koenig). *Int. Res. J. Pharma*. 2(9): 192-193.

Chakrabarty J, Sharma GD and Tamang JP (2009). Substrate utilisation in traditional fermentation technology Practiced by Tribes of North Cachar Hills District of Assam. *Assam University J. Sci. Technol. Biol. Sci.* 4 (I): 66-72.

Chakraborty A and Brantner AH (1999). Antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. *J. Ethnopharmacol.* 68: 339-344.

Cheikhyoussef A, Shapi M, Matengu K and Ashekele HM (2011). Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *J. Ethnobiol. Ethnomed.* 7: 10.

Chougale AD, Padul MV, Arfeen MS and Kakad SL (2009). Antibacterial activity directed fractionation of *Woodfordia fruticosa* Kurz. Leaves. *J. Med. Plants.* 8(31): 75-81.

Das AJ and Deka SC (2012). Fermented foods and beverages of the North-East India. *Int. Food Res. J.* 19**(**2): 377-392.

Das AJ, Deka SC and Miyaji T (2012). Methodology of rice beer preparation and various plant materials used in starter culture preparation by some tribal communities of North-East India: A survey. *Int. Food Res. J.* 19**(**1): 101-107.

Deka D and Sarma GC (2010). Traditionally used herbs in the preparation of rice-beer by the Rabha tribe of Goalpara district, Assam. *Ind. J. Tradict. Knowl.* 5(2): 184-190.

Deori C, Begum SS and Mao AA (2007). Ethnobotany of *Sujen:* A local rice beer of *Deori* tribe of Assam. *Ind. J. Tradict. Knowl.* 6(1): 121-125.

Deshmukh SR, Ashrit DS and Patil BA (2012). Extraction and evaluation of indole alkaloids from *Rauwolfia serpentina* for their antimicrobial and antiproliferative activities. *Int. J. Pharmacy Pharma. Sci.* 4(5): 329-344.

Deshwal VK and Vig K (2012). Study on the antibacterial effect of *Rauwolfia serpentina* on *Staphylococcus aureus*. *Int. J. Pharma. Inv.* 2(7): 45-50.

Dhal NK, Pattanaik C and Reddy S (2010). *Bakhar* starch fermentation, A common tribal practice in Orissa. *Ind. J. Tradit. Knowl.* 9(2): 279-281.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of the plant extracts for bacteria. *Planta Med.* 64: 711-3.

Finose A and Devaki K (2011). Phytochemical and chromatographic studies in the flowers of *Woodfordia fruticosa* (L) Kurz. *Asian J. Plant Sci. Res.* 1(3): 81-5.

Ghosh C and Das AP (2004). Preparation of rice beer by the tribal inhabitants of tea gardens in Terai of West Bengal. *Ind. J. Tradit. Knowl.* 3(4): 373-2.

Gogai B, Dutta M and Mondal P (2013). Various Ethno Medicinal Plants used in the Preparation of *Apong*, a Traditional Beverage use by Mising Tribe of upper Assam. J. Appl. Pharma. Sci. 3(4): S85-S88.

Gopalkrishnan B and Shimpi SN (2012). Pharmacogonostical studies on stem bark of *Madhuca longiflia* (Koeng.) Macbr. Var latifolia (Roxb.) A. *Chavel. Ind. J. Nat. Prod. Res.* 3(2): 232-236.

Gyawali R, Jnawali D, Chang-Hun S and Kim KS (2012). Evaluation of the phytochemical profile and uterine contractile effect of *Woodfordia fruticosa* (L.) Kurz and *Dipsacus mitis* D. Don. *J. Nepal Pharma. Assoc.* 26(1): 1-11.

Harisaranraj R, Suresh K and Saravanababu S (2009). Evaluation of the chemical composition *Rauwolfia serpentina* and *Ephedra vulgaris*. *Adv. Bio. Res.* 3(5-6): 174-178.

Hesseltine CW (1979). Some important fermented foods of Mid-Asia, the Middle-East and Africa. *J. Am. Oil Chem. Soc.* 56: 367-374.

Hooda V, Sharma GN and Singla RK (2011). *Smilax zeylanica* Linn.-A natural therapeutic herb. *Pharmaco Newsletter*. 2: 151-154.

Jhuma S, Bhattacharya S and Rana AC (2012). Phytochemical investigation and pharmaco-gnostic standardization of *Cissampelos pareira* flower. *Int. J. Drug Discov. Herb Res.* 2(2): 442-445.

Kamalakannan P, Kavitha R, Elamathi R, Deepa T and Sridhar S (2012). Study of phytochemical and antimicrobial potential of methanol and aqueous extracts of aerial parts of *Elephantopus scaber* Linn. *Int. J. Curr. Pharma. Res.* 4(1): 18-21.

Kardong D, Deori K, Sood K, Yadav RNS, Bora TC and Gogai BK (2012). Evaluation of Nutritional and Biochemical aspects of Po:ro apong (Saimod) - A home made alcoholic rice beverage of Mising tribe of Assam, India. *Ind. J. Tradict. Knowl.* 11(3): 499-504.

Khatun S, Pervin F, Kari MR, Ashraduzzaman M and Rosma A (2012). Phytochemical screening and antimicrobial activity of *Coccinia cordifolia* L. plant. *Pak. J. Pharm. Sci.* 25(4): 757-761.

Kirtikar KR, Basu BD and An ICG (2005). *Indian medicinal plants*, Vol. II. Edited by Blatter E, Caius JF and Mhaskar KS. 2nd edition, International Book Distributors, Dehradun, India.

Kirtikar KR, Basu BD and An ICG (2005). *Indian medicinal plants*, Vol. I. Edited by Blatter E, Caius JF and Mhaskar KS. 2nd edition, International Book Distributors, Dehradun, India.

Kirtikar KR, Basu BD and An ICG (2007). *Indian medicinal plants*, Vol. IV. Edited by Blatter E, Caius JF and Mhaskar KS. 2nd edition, International Book Distributors, Dehradun, India.

Kirtikar KR, Basu BD and An ICG. *Indian medicinal plants*, Vol. III. Edited by Blatter E, Caius JF and Mhaskar KS. 2nd edition, (International Book Distributors, Dehradun, India), 2005.

Kumar SS, Perumal P and Suresh B (2004). Antibacterial studies on leaf extract of *Elephantopus scaber* Linn. *Anc. Sci. Life.* 23(3): 6-8.

Kumar V and Rao RR (2001). Some interesting indigenous beverages among the tribals of Central India. *Ind. J. Tradict. Knowl.* 6(1): 141-143.

Kumar V and Rao RR (2007). Some plant beverages used in traditional medicines, *Ethnobotany,* 13: 36-39.

Kumaraswamy MV, Kavitha HU and Satish S (2008). Antibacterial potential of extracts of *Woodferdia fruticosa* Kurz on human pathogens. *World J. Med. Sci.* 3(2): 93-96.

Mahomoodally MF, Subratty AH, Gurib-Fakim A, Choudhary MI and Khan SN (2012). Traditional medicinal herbs and food plants have the potential to inhibit key carbohydrate hydrolyzing enzymes *in vitro* and reduce postprandial blood glucose peaks *in vivo. Sci. World J.* 1: 1-9.

Mandal SC, Nandy A, Pal M and Saha BP (2000). Evaluation of antibacterial activity of *Asparagus racemosus* Willd. root. *Phytother Res.* 14: 118-119.

Naik D (1998). Tribal culture in the context of Similipal, *Workshop J*, (District Environment Society, Mayurbhanj, Odisha, India), 63-64.

Nayar SL, Chopra RN and Chopra IC (1956). *Glossary of Indian medicinal plants,* (PID, CSIR, New Delhi), 1956.

Panda SK, Dutta SK and Bastia AK (2010). Antibacterial activity of *Croton roxburghii* Balak.
against the enteric pathogens. *J. Adv. Pharma. Tech. Res.* 1(4): 388-391.

Panda SK, Rout SD, Mishra N and Panda T (2011). Phytotherapy and traditional knowledge of tribal communities of Mayurbhanj district, Orissa, India, *J. Pharmaco. Phytother.* 3(7): 101-113.

Parekh J and Chanda S (2007). *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. flower (lythraceae). *Braz. J. Microbiol.* 38: 204-207.

Poli A, Nicolau M, Simoesb CMO, Nicolau RMR and Zanin M (1992). Preliminary pharmacologic evaluation of crude whole plant extracts of *Elephantopus scaber.* Part I: *in vivo* studies. *J. Ethnopharmacol.* 37: 71-76.

Poovendran P, Kalaigandhi V and Poogunran E (2011). *In vitro* antimicrobial activity of *Coccinia grandis* on ulcer producing *Heliobacter pylori*. *Eur. J. Appl. Sci.* 3(3): 71-74.

Prasad MP, Sushant S and Chikkaswamy BK (2012). Phytochemical analysis, antioxidant potential, antibacterial activity and molecular characterization of *Clerodendrum* species. *Int. J. Mol. Biol.* 3(3): 71-76.

Preethi R, Devanathan VV and Loganathan M (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Adv. Bio. Res.* 4(2): 122-125.

Rout SD and Panda SK (2010). Ethno-medicinal plants resources of Mayurbhanj District, Orissa, *Ind. J. Tradit. Knowl.* 9(1): 68-72.

Roy JK (1978). Alcoholic beverages in tribal India and their nutritional role. *Man India,* 58: 298-326.

Sahu TR (1996). Life support promising food plants among aboriginals of Bastar (MP), India, In: *Ethnobotany in human welfare*, edited by SK Jain, (Deep publications, New Delhi), pp. 26-30.

Singh BH (1996). Ethnobotanical observation on the preparation of *Choarak* (a local wine) in Tripura state. *J. Eco. Taxon Bot.* 12: 373-278.

Singh PK and Singh KI (2006). Traditional alcoholic beverage, *Yu* of *Meitei* communities of Manipur. *Ind. J. Tradict. Knowl.* 5(2): 184-190.

Steinkraus KH (1994). Nutritional significance of fermented foods, *Food Res. Int.* 27: 259.

Syed Z, Krishna B, Kandukuri V and Singara C (2009). Antimicrobial activity of the fruit extracts of *Coccinia indica*. *Afr. J. Biotechnol.* 8(24): 7073-7076.

Tamang JP (1992). Studies on the microflora of some traditional fermented foods of the Darjeeling hills and Sikkim. Ph.D. thesis, North Bengal University, Darjeeling.

Tamang JP (1998). Role of microorganisms in traditional fermented food. Ind. Food Industry, 17(3): 162-166.

Tamang JP and Sarkar PK (1995). Microbiology of *murcha*: An amylolytic fermentation starter. *Microbios,* 81: 115-122.

Tamang JP and Sarkar PK (1998). Traditional fermented Food and beverage of Darjeeling and Sikkim - a Review. J. Sci. Food Agric. 44: 375-385.

Tambekar DH and Khante BS (2011). Antibacterial evaluation of medicinal plants used by Korkus in Melghat forest against gastrointestinal infections. *Int. J. Pharma. Sci. Res.* 2(3): 577-583.

Tamilselva N, Thirumalai T, Elumalai EK, Balaji R and David E (2011). Pharmacognosy of *Coccinia grandis*: a review. *Asia Pacific J. Trop. Biomed.* S299-S302.

Tanti B, Gurung L, Sarma HK and Buragohain AK (2010). Ethanobotany of starter culture used in alcohol fermentation by a few ethnic tribes of Northeast India. *Ind. J. Tradict. Knowl.* 9 (3): 463-466.

Thatoi HN, Panda SK, Rath SK and Dutta SK (2008). Antimicrobial activity and ethnomedicinal uses of some medicinal plants from Similipal Biosphere Reserve, Orissa, *Asian J. Plant Sci*. 7 (3): 260-267.

Tiwari SC and Mahanta D (2007). Ethnological observations fermented food products of certain tribes of Arunachal Pradesh. *Ind. J. Tradict. Knowl.* 6(1): 106-110.

Trease GE and Evans WC (1989). *Text book of Pharmocognosy*. 3rd ed. London: Bailliere Tindal.

Tsuyoshi N, Fudou R, Yamanaka S, Kozaki M, Tamang N, Thapa S and Tamang JP (2005). Identification of yeast strains isolated from *marcha* in Sikkim, a microbial starter for amylolytic fermentation. *Int. J. Food Microbiol.* 99 (2): 135-146.

Umamaheswari M and Chatterjee TK (2008). *In vitro* antioxidant activities of the fractions of *Coccinia grandis* leaf extract. *Afr. J. Tradit. Compl. Alter. Med.* 5(1): 61-73.

Vidya SM, Krishna V, Manjunatha BK and Raghavendra RB (2010). Antibacterial activity of *Clerodendrum serratum* L. *Elec. J. Env. Agric. Food Chem.* 9: 1-10.

Wani JA, Achur RN and Nema RK (2011). Phytochemical screening and aphrodisiac activity of *Asparagus racemosus. Int. J. Pharma. Sci. Drug Res.* 3(2): 112-115.

Yokotsuka T (1991). Non-proteinaceous fermented foods and beverages produced with *koji* molds. In: *Handbook of Applied Mycology*, edited by DK Arora, KG Mukherji and EH Marth, (Marcel Dekker Inc. New York), 3: 293.

CHAPTER SIX

USE OF PLANT EXTRACTS IN PLANT DISEASE MANAGEMENT: ROLE AND SIGNIFICANCE OF CHARACTERIZATION OF SECONDARY METABOLITES FROM HIGHER PLANTS IN PHYTO-DISEASE MANAGEMENT

Enyiukwu, D N., Awurum, A. N., Ononju, C. C and Nwaneri, J.
Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, P.M.B 7269 Umuahia, Abia State Nigeria.

ABSTRACT

Several sources reported the advantages of synthetic fungicides in boosting food production. However, there are serious concerns and negative consequences associated with their use on ecological and human health in recent years. Besides, pathogens' resistances to some of the most effective fungicides have been reported. These amongst others prompted search for alternatives. Plants offer an expanse of exploitable chemical space in this regard which is unparalleled in nature and not beaten by combinatorial chemistry as yet.Extracts of higher plants have demonstrated a wide range of activity against plant pathogenic organisms. These plants extracts have been found to contain broad spectra of phytochemicals (secondary metabolites) such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, terpenoids, phlobatannins, polyphenols and steroids. Secondary metabolites constitute plants' weaponry against pest and pathogen invasion. These groups of phytochemicals possess wide ranging chemical functional groups; by which they establish and bind to sites on target pathogens to ineffectuate them. Complexes of these chemical compounds occur and incrude extracts. Need therefore arises in the present to identify, isolate and purify the active principle(s) in the extracts and to determine theone(s) effecting the reported kills and fungitoxity of the extracts. With the exception of neem assertive reports on their modes of action are unavailable. We have to understand the functional groups of the isolates, their position on the carbon skeleton of the principles, binding sites on target pathogenic species; and the metabolic process(es) which they affect and ineffectuate. This will aid in either synergizing or synthesizing them so as to combat the enormous and obvious challenges of fungicide resistance threating agricultural production and food security. This work reviews available literature in these regards.

***KEYWORDS**: Plant extracts, secondary metabolites, Plant disease, chemical characterization, MOA*

INTRODUCTION

The National Bureau of Statistics (NBS) of the Government of Nigeria listed the important field crops grown in Nigeria. These crops include yam, cocoyam, cassava, cowpea, groundnut, sorghum, millet, maize, rice, melon and cotton. According to Abate *et al*. (2011), FAOSTAT also included soybean as an emerging important field cropalso grown inNigeria. Statistics show that Nigeria is the world's largest producer of yam accounting for 70-76% of the world production. In 2008 for instance, Nigeria produced 35.017 million MT of yam valued at US$ 654 billion,on 3.045 million ha of farmlands (Kleih *et al.,* 2012; Wikipedia, 2012). FAO (2006) on the other hand reported that Nigeria is the largest producer of cocoyam in the world, with 5.49 million tonnes of cormsproduced annually which accounts for 37-40% of the world's total. Similarly, statistics reveal furtherthat out of the 29.5 million MT of groundnut produced worldwideper annum, Nigeria alone contributes 1.51-2.57million MTproduced on 2.09 million ha; similarly,0.53 million MT of soybean werereported produced from0.582 million ha of arable farmlands respectively (Abate *et al.* 2011, Soytech, 2011; Awurum and Uwajimgba, 2013). She is also the world's largest producer of the grain legume, cowpea. Furthermore, Singh *et al*. (2002) noted that Nigeria produced 2.0 million MT of cowpea grains on 5 million ha per annum.Many socio-economic factors, pests and disease pressures however, threaten the sustainableproduction, storage and preservation of these staple foods as well as several leafy vegetables, fruits and spicesamongst others in the country (Awurum *et al* 2000; Abate *et al*., 2012); and these threats are projected to increase in the face of climate change challenges (Sadiku and Sadiku, 2011).

Fungi represent the greatest number of pathogens responsible for these plant diseases and deteriorations (Ajibade and Amusa, 2001). Many species of fungi decimate agricultural crops or products in field, transit or store. It has been reported that up to 8000 – 750,000 crop diseases are caused by fungi with between 50 – 200 species, races and/or biotypes attacking a single crop (Ragsdale, 1994; Madden *et al*., 2008). Infection by fungi interferes with normal physiological function(s) of the host plant such as photosynthesis, biosynthesis, nutrient and water uptake among others, leading to great reductions in crop yield and product quality. Some pathogenic fungi such as *Aspergillus spp. and Fusarium moniliforme*produce toxins – ***mycotoxins*** – which cause cancer or may be nerve or organ poisons, making affected produce dangerous for human and livestock consumption (Ragsdale, 1994). The incidence of these plant diseases upon a susceptible host is dependent on the presence of specific conducive conditions of moistures in the atmosphere, in soil and/or on plant surfaces; in addition to optimum temperatures for infection and disease development (Ragsdale, 1994; Amadioha, 2012).
Field phyto-fungal diseases may be nematode-assisted. Nematodes of the order *Xiphenima, Trichoderma, Longidorus and Para-longidorus* have been implicated in this regard. These stylet-possessing organisms puncture and burrow into tender, succulent

plant roots sucking out vital growth factors from the infested crop-plants, creating portals of entry for pathogenic fungi and bacteria; reducing plant anchorage and vigour, and predisposing the affected crop to damaging infections. For example the yam nematode *Scutellonema bradys* and species of *Melodogyne* have been noted to attack yam in Southeast Nigeria, cowpea isseriously galled by members of the genera *Meloidogyne*while *Practylenchus spp.* attack plantains and bananas in the rainforest zone of the country (Wikipedia, 2013).

Emechebe and Shoyinka (1985) reported that pathogenic fungi are capable of causing up to 100% yield loss in crops. Worldwide, it is projected that 20 – 40% of potential yield of crops are lost annually to plant diseases (Sygenta, 2012). This may aggravate to 50%, particularly in Africa due to climate change impacts, noted Sadiku and Sadiku (2011).It is estimated that globally these yield losses amounts tobetween 60 – 525 billion US dollars annually (Agrois, 2004; Sygenta, 2012). According to Amadioha (2012), in Nigeria alone, Nkama *et al.* (1994) estimated that 1.5 – 20 million tonnes of cereals, tubers and legumes amounting to 0.3 – 0.4 billion Naira are lost annually in storage due to fungal attacks. In a bid to control these devastating attacks, synthetic fungicides are employed.Without controversy, fungicides contribute to yield increases in crop production. Adipala and Edema (1994) reported that the application of Mancozeb and Dithane M-45 significantly improved the yield of cowpeas in Uganda.In the USA noted Bernnett (2005), 1.5 billion pounds of onion was harvested from onion plants cared for with 0.8 million pounds of fungicides. Economic use of fungicides in agriculture translates to improved returns on farm investment. According to this source, in California apple growers invested 70 million dollars on various fungicides, and reaped an attractive 1.2 billion dollars while grape growers got 2.6 billion dollars as return on investment (ROI) of 128 million dollars in the same period. A total of 880 million dollar-investment in fungicides by growers in the USA all together reaped the sum of 12.8 billion dollars ROI translating to US$1 fungicides investment to US$14.6financial returns for them. Fungicides play a very active role in production of high value crops with uniform appearances and quality (Biobank, 2009). Highly intensive and developed crop farming as practiced in the USA and Europe, involves use of highly-bred crop varieties to maintain uniform crop height, canopy, fruit size and shape as well as overall appearance and quality of produce in mechanized farms. Without fungicides and other pesticides it will be difficult to grow such crops ofhigh horticultural characteristics in large monocultures given serious potential pathogenic challenges in the environment. In the USA, concluded Croplife (2012) many crops would not be produced commercially without fungicidal agents.

However, the high intensity of chemical pesticide applications and/or their inappropriate applications in agriculture have become a serious cause of concern in recent years reported Biopesticides (2012). Several demerits are obviouslyassociated

with use of these synthetic fungicides in agriculture and pest controlhave been reported such as pathogen resistance, pathogen resurgence, effects on non-target species, ecological and human health concerns among others. Resistance to chemical agents is a very serious matter. Recently FAO according to Par and Rajul (1994) noted 150 fungal pathogens to exhibit resistance to fungicides. Some authorities assert that evolution of races and biotypes of pathogens to previously effective chemical agents occur 5-10 years after introduction of the agent (Oreason, 2010; Pallant, 2010).Besides, synthetic pesticides leave undesirable residues in the treated food materials and the environment. Some of these residues retain their toxic properties for a long time in the food chain, impairing metabolic processes when consumed by non-target species. These and many other factors gave impetus for alternatives to be sought (Awurum *et al*., 2005; Okwu *et al*., 2007; Amadioha, 2012).Some of the preferred alternatives are:

- Use of natural enemies of the pathogen such as *Trichoderma sp., Gliocladium sp., Bacillus thurengiensis* and *Baculoviruses* in managing challenges from the pathogen (Carson's Silent Spring, 1963).
- Use of Intercropping to forestall and manage pests and pathogens problems in smallholder farms (Awurum *et al*., 2001).
- Farming systems re-design, through adopting crop rotation practices, proper field sanitation, good crop density, improved field aeration, and furrow instead of overhead sprinkler irrigation systems etc., so as to reduce predisposing crops to fungal attacks (Abawi and Hunter, 1979; Anonymous, 2012).
- Use of botanical pesticides such as neem (Azatin, Bioneem, Tomco and Mangosan)and extracts of other higher plants to combat challenges from phyto-pathogenic organisms (Amadioha and Obi, 1998; Amadioha, 2002; 2003; 2004; Awurum *et al*., 2005)

This review focuses on use of botanical pesticides in plant health management. Many higher plants of the rain forest are being screened for fungicidal properties (Amadioha. 2001; 2002; 2003). Several workers have conclusively asserted the fungitoxic properties of these plant-based extracts for management of phyto-fungal diseases (Olufolaji, 1999; Awurum *et al*., 2005; Opara and Obana, 2010). These extracts have been reported to have the merits of being readily available in faming localities of the tropics, cheap, eco-compatible, less harmful to non-target organisms and useable in Integrated Disease Management (IDM) programmes for smallholder resurce-poor farmers. They are also reported to provide sustainable disease management solutions especially in organic farming where synthetic pesticides are non-tolerable. Above all, they are systemic and contain multiple bioactive metabolites which make pathogens' resistance to them less likely (Adjaye-Gbewonyo *et al*., 2010; Opara and Obana, 2010; Gurjar *et al*., 2012).

USE OF PLANT EXTRACTS IN PLANT DISEASE CONTROL

According to Cowan (1999), initial screening of plants for antimicrobial activities; begin with their crude aqueous or alcoholic extracts.Literatureavailable to us indicated thatextracts of wild *Ipomea carnea* and isolates of *Penicillin sp*. had fungitoxic effects against *Heminsthoporium oryzae*, *H. sativum* and *Colletotrichum capsici* which affect paddy rice, wheat and chilli respectively (Narian, 1970). Many plant extracts as pesticides have been reported to inhibit spore germination and mycelial growth of pathogenic fungi (Amadioha and Obi, 1998; Olufolaji, 1999).These crude extracts were evaluated *in vitro* for activities against germination of the spores and mycelial growth of the pathogens, and later, for *in vivo* inhibition of the development and spread of the organisms in actual field conditions.Gurjar *et al.* (2012) however argued that majority of these works on extract evaluations against pathogenic organisms were conducted*in vitro*. The use of plant extracts in plant protection is summarized in Table 1. They have been found effective against seed-inhabiting pathogens, soil dwelling, nutrient and water uptake impairing organisms such as the wilt-inducing *Fusariumoxysporium* of egg plants and rootknot nematode (*Meloidogyne spp*.) of okra as well as rots and other phyto-fungal pathogens of*Amaranthus*, legumes, tomato, yam, and avocado etc. (Amadioha and Obi, 1998; Olufolaji, 1999; Amadioha, 2001; 2003). They have also proven effective for arresting the development and spread of bacteria-induced diseases of vegetables and tuber crops. Several plant extracts have been evaluated and found efficacious against pre- and post-emergence damping-off, post-harvest rot and transit-decay inciting pathogens of great implications in threating post-harvest storage and preservation of produce in agriculture such as species of *Diplodia, Aspergillus, Botrytis, Botrydiplodia, pythium, Fusarium, mucor, Rhizopus, Penicillin, Sclerotinia, Alternaria ,Rhizoctonia and phytophtora* (Okigbo and Ogbonnaya 2006; Sarpeleh *et al.,* 2009; Gupta *et al*., 2012; Islam and Faraq, 2012). For example, recently phytochemicals from some tropical plants (*Carica papaya* and *Piper guineense*) strongly retarded the germination of spore of*Colletotrichum destructivum* (Enyiukwu and Awurum,2012).Okigbo *et al*. (2012) reported the isolation of*Botrydiplodia theobromae* as the most virulent amongst other pathogenic rot fungi from cocoyam corms in Southeast Nigeria and showed that extracts from*Allium sativum*and*Azadirachta indica* were the most fungitoxic against them. Similarly, Amienyo and Ataga (2007)indicated also that 30% strength of extracts of *Alchornea cordifolia*leaves reduced development of rot in mechanically injured and artificially inoculated sweet potato by the same organism to the tune of 46%. In an evaluation in sorghum, *Cymbopogoncitratus*(30% strength) completely inhibited the growth of *Colletotrichum graminicola* and *Phoma sorghomi* causing seed and seedling rot in the plant (Somda and Sereme, 2007). Gupta *etal*. (2012) reported that extracts from *Eucalyptus terticornis* and *A. indica* improved seed germination and seedling vigour by decreasing the pre and post-emergence mortality and number of seedlings showing symptoms of black mould in attacked onion.Greenhouse studies conducted in Southeast Nigeria revealed that *Piper guineense* and *Carica papaya* extracts inhibited the

development and spread of anthracnose caused by *C. destructivum* and the results compared well with a placebo. In field trials, Awurum and Nwaneri(2011) and Awurum and Ogbonna (2013) reported extracts from *Dennettia tripetala* and *Spondias mombin* comparable in fungitoxic effects to benomyl in combating *Choanephora cucurbitarium*-induced wet rot of *Amaranthus* vegetable.On-farm research depicted that while in Cameroun, Ambang *et al*. (2011) found methanoic extracts of *Thevetiaperuviana* effective in controlling cercospora leaf spot (CLS) in groundnut, Awurum and Uwajimgba also found *Dennettia tripetala* fungicidal against *Fusarium* wilt of the same crop; and both reports compared well with benomyl treated plants. In Kenya, experiments on-farm on Common bean (*Phaseolus vulgaris L.*), indicated that neem inhibited *Fusarium spp*. in amended soils better than tobacco, Mexican marigold and periwinkle (Obongoya *et al*., 2010). There is something to note here however that in soil amendment trials, combinations of extracts involving 500 extracts from different families/genera showed antagonistic, synergistic and neutral effects. Glove and black pepper (1% concentration) combination for example, was found synergistic against mycelial growth and sclerotialformation of the pathogen *Sclerotium cepivorium* while antagonism was observed in all extract combination involving allspice, and clove and cinnamon at 0-3% against the organism (Montes-Belmont and Prados-Ligero, 2006).Theseevaluations in general clearly demonstrated that the plant extracts from a wide range of families are potently efficacious in cultures, glasshouse studies and in field trials in inhibiting mycelial growth of diverse organisms,arresting their spore germinationanddevelopment as well as spread of pathogenic diseases from both artificially and naturally infected plants in actual field conditions.

Table 1: Some important phyto-diseases controlled with extracts of higher plants

DISEASE	CROP	PATHOGEN	EXTRACT USED	SOURCE
Bacterial blight	Egg plant	*Xanthomonas campestris pv vesicatoris*	*Piper guineense,* *Zingiber sp.,* *Allium sp.*	Opara and Obana, 2010 Opara and Wokocha, 2008.
Anthracnose	Cowpea	*Colletotrichum destructivum*	*Piper guineense seed,* *Carica papaya root, seed*	Enyiukwu and Awurum (2011; 2012; 2013)
Wet rot	*Amaranthus*	*Choanephora cucurbitarium*	*Azadirachta indica bark.*	Olufolaji, 1999. Awurum and

			Dennetia tripetala leaf,	Nwaneri, 2011.
			Spondias mombin leaf	
Sclerotium stem rot	Cowpea	*Sclerotium rolfsii*	*Afromonium meleguata seed*	Okwu and Njoku, 2009
			Monodora myristica seed	
Stem rot	Cowpea	*Rhizoctonia solani*	*Piper guineense leaf,*	Amadioha (2001; 2012)
			Carica citratus leaf,	
			Ocimum sp. Leaf.	
			Ocimum sanctum.	
Blast	Rice	*Pyricularia oryzae*	*Azadirachta indica seed oil.*	Amadioha (2000; 2012)
Brown spot	Rice	*Cochlobolus miyabeanus*	*Azadirachta indica (ethanolic extract).*	Amadioha, 2002
Black pod	Cocoa	*Phytophtora palmivora*	*Carica papaya seed*	Wokocha and Nwogu, 2008.
			Garcinia kola seed.	
Mould	Mung bean	*Aspergillus niger*	*Vernonia sp. Leaf*	Onuegbu, 1996.
(Black)	Onion	*Aspergillus niger*	*Eucalyptus terticornia*	Gupta *et al*., 2012
			Azadirachta indica	
Basal stem rot	Tomato,	*Sclerotium rolfsii*	*Garcinia kola,*	Wokocha and Okereke, 2005.
			Hyptis spp.	
	Cowpea		*Citrus peel.*	Okwu *et al*; 2007
		Curvularia	*Datura stramonium root,*	

		lunata	*Colotropis procera stem,* *Ocimum sanctum Leaf*	
Stem rot	Vanilla	*Fusarium oxysporium f. sp. vanilla*	*Eugenia aromatica leaf,* *Piper bettle leaf,* *Alpinia galangal rhizome,* *Sphaeranthus indica leaf.*	Suprita and Khalim, 2009.
Rot	Cashew-nut	*Aspergillus spp.,* *Trichodrema spp.,* *Cephalosporium sp.*	*Anacardium occidentalis leaf* *Vernonia amygdalina leaf.*	Suleman and Ogundana, 2010.
	Musk bean, Cucumber	*Phytophtora drechsleri* *Verticillium diahlae* *Scleretina scleroiorium* *Aternaria sp.* *Botrytis cinerea* *Macrophomina phaseolus*	*Perganum hamala* *(shoot, flower, seed)*	Sarpeleh *et al.*, 2009.
Root rot	Cowpea	*Pythium alphanidermat*	*Aloe vera leaf,*	Suleman and Emma, 2009.

		um	*Garcinia kola seed,*	
			Azadirachta indica,	
			Zzingiber officinale	
	Cowpea		*Garcinia kola*	Suleiman and Emua, 2009.
	Maize/tomato	*Pythium aphanidermatum*	*Ziginber officinale*	
			Hermidesmus indicus	Sangrikar and Wadje, 2012.
		Alternaria solani	*Withania sominifera*	
			Rauwolfia tetraphylla.	
		Fusarium moniliforme		
Post-harvest rot	Yam		*Ocimum gratissimum leaf,*	Okigbo and Ogbonna, 2006.
			Afromonium meleguata.	
		Aspergillus niger	*Xylopia aethiopica*	Okigbo and Nmeka, 2005.
		Aspergillus flavus	*Zingiber officinale*	
	Cassava	*Fusarium oxysporium*	*Afromonium meleguata seeds*	Okigbo *et al.*, 2009.
		Botrydiplodia theobromae	*Azadirachta indica*	
		Fusarium		Ugwuoke *et al.*,

	Cocoyam	*solani*		2008.
		Penicillin oxalicum	*Ocimum basilium* *Vernonia amygdalina* *Azadirachta indica*	
		Geotrichum candida		
		Cortichum rolfsii		
		Sclerotium rolfsii		
		Aspergillus niger		
		B. theobromae		
		F, oxysporium		
Cercospora leafspot	Cowpea	*Cercospora spp.*	*Dennettia tripetala*	Nwachukwu, 2010
Curvularia leaf spot	Maize	*Curvularia lunata*	*Phyllanthus amarus* *Tithonia diversifolia* *Morinda lucida* *Gliricidia sepium*	Akinbode, 2010
Damping off	Cowpea	*Scleretium rolfsii*	*Annona municata*	Chukwu, 2010.
	Eggplant, Chilli pepper,	*Rhizoctonia*	*Azadirachta indica* *Allium sativum*	Islam and

	Tomato	*solani* *Fusarium oxysporium* *Sclerotium rolfsii*	*Zingiber officinale* *Allamonde leaf*	Faraq, 2012.
Rice blast	Rice	*Pyricularia oryzae*	*Chloranthus japonica roots,* *Paulonia coreana stem*	Choi *et al.*, 2004.
Rootknot nematode	Okra	*Meloidogyne spp.*	*Azadirachta indica*	Asaawalam and Adesanya, 2001.
Dry rot	Yam	*Fusarium oxysporium,* *Aspergillus niger.*	*Aloe babadensis leaf,* *Nicotinia tabacum leaf,* *Azadirachta indica leaf.*	Taiga, 2009
Seed rot (dry)	Melon	*Rhizopus stolonifer*	*Ocimum gratissimum* *Azadirachta indica*	Chuku *et al.*, 2010.
	Sorghum	*Penicillium italicum* *Aspergillus niger* *Colletotrichum gramminis* *Phoma sorghumi*	*Cymbopogon citratus* *Eucalyptus camaldulensis*	Somda and Serene, 2009.

		Fusarium moniliforme		
	Potato/ Vegetables	*Rhizoctonia bataticola* *Rhizoctonia solani* *Aspergillus flavus* *Aspergillus niger*	*Aloe vera* *Azadiractha indica* *Ocimum sanctum*	Gurjar and Telwankar, 2012
Brown spot	Rice	*Bipolaris oryzae*	*Nerium oleander leaf*	Harish*et al.*, 2008.
			Callistemon citrinus *Ocimum gratissimum.*	Nguefack *et al.*, 2007
Fruit rot	Pawpaw	*Aspergillus niger* *Botrydiplodia theobromae*	*Carica papaya* *Chromolaena odorantum* *Acalypha ciliate*	Ilondu, 2011
White rot	Onion	*Fusarium solani* *Penicillin sp.* *Sclrerotium ceporium*	*Pimenta dioica* *Syzygium aromaticum* *Piper nigrum*	Montes-Belmont and Prados-Ligero, 2006
Wilt	Brijal	*Fusarium oysporium f. sp. Melongae*	*Azadirachta indica* *Artemisia annua* *Eucalyptus glabulus*	Babu *et al.*, 2008.

			Ocimum sanctum *Rheum emodi*	
	Tomato	*Fusarium oxysporium f. sp. lycopersici*	*Azadirachta indica kernel, stem, leaf*	Agbenin and Marley, 2006.
Brown blight	Tea	*Glomerella cingulate*	*Pongamia pinnata* *Syzigium aromatia* *Alcorous calamus* *Ageratum conyzoides* *Allium sativum* *Abutilon indicus*	Kuberan *et al.*, 2012
Blight	Wheat	*Bipolaris sorokiniana*	*Adhatoda vasicola* *Zingiber officinale*	Nagis *et al.*, 2012
Seed-borne diseases*(rot, seedling blight, Bipolaris leaf spot, curvularia leaf spot)*	Maize	*Fusarium oxysporium* *Fusarium moniliforme* *Penicillin spp* *Aspergillus spp.* *Bipolaris maydis* *Curvularia*	*Allium sativum* *Azadirachta indica*	Debnath *et al.*, 2012

		lunata *Rhizoctonia stolonifer*		
Seed/seedling rot	Soybean	*Cephalosporium acromonium* *Rhizopus leguminosa* *Colletotrichum dermatium* *Macrophomina phaseolina* *Phoma sp.* *Sclerotium rolfsii* *Curvularia lunata* *Fusarium oxysporium* *Penicillin chrysogenum* *Mucor mucedo* *Alternaria alternate* *Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus*	*Acacia nilotica* *Azadirachta indica* *Datura stramonium* *Polyathia longifolia* *Annna squamosal* *Allium sativum*	Rathod and Pawar, 2012.

Plants extracts as seen from Table 1, are hence suitable for exploitation as potent sources of pesticides to reduce losses arising from pathogenic attacks on crops and stored products (Amadioha, 2012). The use of these natural products for pathogenic disease management is particularly important and necessary in the developing economies of the world like Nigeria where synthetic fungicides are not only unavailable but farmers who produce about 98% of food in the country are poorly equipped to handle them making their use uneconomic for resource-poor farmers.

Phytochemical evaluation of extracts

It has been noted that, despite the use of plant extracts in ethno-medicine, African cuisines and recently in plant protection, that their phytochemical composition and active ingredients have notbeen fully documented (Okwu and Njoku, 2009). Corroborative studies estimated that only 4% -10% of the 250,000 plant species constituting the biodiversity of the world's flora have been examined chemically for antimicrobial activity (Earnsworth, 1990; Pallant, 2010). Ahuge potential therefore exists in this regard; especially in the tropical areas like Nigeria with vast untapped rainforest, for these fungitoxic plant species to be extensively examined chemically. Scientific analysis of plants it has been observed follows a logical pathway beginning with a lead from the natives. Hence, Cowan (1999) reported further, that initial aqueous and alcoholic screening of plants extracts for antimicrobial activities are followed by other organic extraction methods for determination of their phytochemical compositions. According to Wessells and Hopsons (1988), marvelous assortments of chemicals which are noxious to pathogens and even pests, have been found to be present in plants. These antimicrobial constituents (phytochemicals) include alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides, anthraquinones, coumarins, polyphenols, Phlobatannin and steroids (Wessells and Hopsons, 1988; Edeoga *et al*., 2005;Okwu and Njoku, 2009; Soladoye and Chukwuma, 2012). The phytochemical constituents of some plant materials used in plant protection are presented in Table 2. They may be qualitatively represented or quantitatively documented (Edeoga *et al*., 2005; Jeruto *et al.,* 2011). In a qualitative evaluation, Ayodele *et al*. (2009) reported the absence of alkaloids and anthraquinones from the leaves, stem bark and root of *Ficus exaspeata*. Quantitative documentation of phytochemicals in plant materials are presented in mg/100g of dry weight of specimen, g/100g of weight of the specimen (Aliyu *et al*., 2008; Harisaranraj *et al*., 2009; Okwu and Iroabuchi, 2009; Senthilkumar *et al*., 2011) or as percentages of weight per volume (w/v) of extract (Edeoga *et al*., 2005; Okwu *et al*., 2007; Uchegbu and Okwu, 2012; Enyiukwu and Awurum, 2013). This review will only present results expressed as percentages. Studies show that sometimes there may be disparity in the reported yield values of these phytochemicals by different

investigators. For instance,Enyiukwu and Awurum (2013) reported 1.63% alkaloids present in the seeds of *Piper guineense* which figure differed hugely from the value 5-8% noted in Purseglove (1976). This difference in values the authors attributed to influences from time of harvesting of the plant materials, extracting solvent, and method of extraction. The latter two factors seem most important to the reviewers. Variation in extracting methods are usually dependent on the length of the extraction period, solvent used, solvent pH, temperature, particle size of plant material and the solvent-to-sample ratio. According to Gurjar *et al*. (2012), the finer the particles size the higher and better the rate of extraction. Furthermore, in a study by (Elloff, 1998) it was found that 5 minutes extraction of very fine particles of diameter 10 nm gave higher quantities of phytochemicals than values obtained after 24 h in a shaking machine with less finely ground materials. Later investigations revealed that solvent-to-sample (solvent to dry weight) ratio of 10:1 had proved ideal for extraction of phytochemicals (Green, 2004; Gurjar *et al.,* 2012).The observed differences in the yield of phytochemicals amongst parts (leaf, stem, bark, flower, seed) of the sameplant, the authors further added may be occasioned by factors of age of the plant, plant part, sex and cultivar used in the investigation. Enyiukwu and Awurum (2013) noted that in a study at Cornell University, that male *Carica* plants yielded more phytochemicals than female ones and older plant parts yielded more than the young ones. Similarly, it was also noted in the samestudy that the potency of activity of the yielded phytochemicals were age of plant or plant part dependent. For instance, phytochemicals from young plants or plant parts were observed to be more active than those from older plants or plant parts.Variations in yield values of phytochemicals may also be occasioned by the climatic and edaphic variations in the geographic locations of growth of the plant (Pallant, 2010).

Phytochemicals of the group alkaloids have complex structure. They are bitter tasting, colourless, basic, and toxic,and contain nitrogen in a heterocyclic ring.At room temperature alkaloids may be liquids or crystalline solids (Okigbo *etal*., 2009). They are the most physiologically active compounds of medical importance found in plants. Alkaloids and their derivatives are used as basic starting points for drugs. They possess antifungal and bactericidal properties (Okwu and Uchendu, 2009). Karlovsky (2008) reported that alkaloids can inactivate enzymes, block ionchannels, interfere withneurotransmission and cause loss of electrical coordination (ataxia) in organisms.

Flavonoids are polyphenolic compounds possessing 15 carbon atoms made up of two benzene rings joined by linear carbon chain. They represent the most common and widely distributed class of plant phenolics. Flavonoids are a class of secondary metabolites known most commonly for their antioxidant and free radicals scavenging activities. Aside of prevention oxidative cell damage, flavonoids also play roles in combating allergies and microbes (Okigbo *et al*., 2009). Some flavonoids of the sub-class isoflavonoids, exhibit high pesticidal activity by anaesthetic-like action related to

electron transport blockade in the mitochondria brought about by inhibiting oxidation linked to $NADH_2$. (Freidli, 2008; Okwu and Njoku, 2009).

Table 2: Phytochemical composition of some plants materials used in plant protection.

	Phytochemical constituents *(%)								
Plant Materials	Alka	Flav	Tan	Sap	Phe	Ter	Gly	Ste	**Source/References**
Ricimus communis leaf	-	+	+	+	+	-	+	+	Yadav and Agarwala, 2011.
Xanthium strumarium leaf	+	+	+	+	+	-	+	-	" " "
Tinospora cordifolia	+	+	+	+	+	+	+	+	" " "
Hyptis sauveolens	+	-	+	+	-	+	+	-	Pachkore *et al*., 2011
Clutiaabyssinica	-	+	-	+	+	+	-	-	
Euphobia hirta	+	+	+	+	+	+	+	-	Ibrahim *et al*., 2012
Eruca sativum	11.27	*24.43*	4.15	6.20	26.90	-	2.76	-	Mohammed *et al*., 2011.
Alchornea cordifolia leaf	5.90	*4.20*	6.80		3.20				Adeshina *et al*., 2012
Cleome rutidosperma	0.34	*0.34*	15.25	2.00	0.20	-	+	-	Edeoga *et al*., 2005
Emillia coccinea	0.92	*0.96*	11.85	2.30	0.81	+	+	+	" "
Euphobia heterophylla	0.86	*0.74*	12.46	0.00	0.10	+	+	-	" "
Physalis angulate	0.40	*0.15*	13.15	3.92	0.80	+	+	+	" "
Richadis brasilienss	0.45	*0.56*	12.13	1.12	0.14	+	+	+	" "

Scorpania dulchis	0.81	*0.88*	6.23	0.00	0.04	+	+	-	" "
Sida acuta	1.04	*0.98*	6.08	0.00	0.08	-	+	-	" "
Spigella anthemia	0.84	*0.77*	15.05	2.26	0.10	-	+	+	" "
Stachytaphyta cayennensis	0.68	*0.00*	9.98	3.10	0.13	-	+	-	" "
Tridax procumbens	0.58	*0.61*	7.45	1.70	0.06	-	-	-	" "
Carica papaya seed	0.62	*0.34*	0.22	0.68	0.08	-	+	-	Enyiukwu and Awurm, 2013
Carica papaya root	0.75	*0.57*	0.34	1.40	0.05	+	+	+	" "
Piper guineense seed	1.63	*1.23*	0.88	2.64	0.66	+	-	+	" "
Monodora myristica leaf	4.28	*8.29*	0.34	0.02	0.03				Okwu and Njoku, 2009
Monodora myristica seed	0.41	*0.12*	0.03	0.87	0.02				" "
Bryophyllum pinnatum	1.48	*1.72*	0.51	1.74	0.06				Okwu and Uchenna, 2009
Cissis populnea root	2.79	*4.13*	1.18	1.11	-	0.36	0.53	0.13	Soladoye and Chukwuma, 2012
C. populnea stem	4.70	*1.46*	1.10	1.21	-	0.27	0.41	0.15	Okwu and Uchenna, 2009
Cassia alata seed	3.24	*0.50*	2.46	6.44	0.95				" "
Nauclea latifolia leaf	4.32	*0.36*	0.01	0.98	0.06				" "

Nauclea latifolia seed	0.59	*0.56*	0.06	1.34	0.05		" "
Nauclea latifolia fruit	0.28	*0.81*	0.01	0.42	0.02		" "
Azadirachta indica leaf	0.52	*0.62*	9.10	2.10	0.02		Khrishnaiah *et al*., 2009
Molinger oleifera	0.36	*0.51*	9.20	2.30	0.08		" "
Cleredendon sp. Leaf	5.41	*0.70*	3.60	2.10	0.08		Okwu and Uchenna, 2009.
Spondias mombin leaf	6.00	*3.00*	3.80	7.60	1.00		Njoku and Amaefula, 2007.
Afromonum meleguata leaf	0.29	2.15	0.16	0.14	0.10		Okwu and Njoku, 2009
Afromonum meleguata seed	5.64	*5.78*	0.39	1.24	0.11		" "
Chromolaena odorantum	-	-	1.98	0.38	-	0.13	Igboh *et al*., 2009.
Citrus limonum	0.54	*0.64*	1.31	0.34	0.25		Okwu *et al*., 2007.
Detarium Senegalese	0.72	*5.68*	0.79	4.60	0.25		Uchegbu and Okwu, 2012.
Uvaria chamae	0.81	*5.70*	0.40	0.38	0.10		Okwu and Iroabuchi, 2009.

*= qualitative representation. + indicate presence of constituent; - indicate absence of constituent.

Tannins comprised of a large assemblage of natural products which have unpleasant taste and are employed in tanning leather (Okigbo *et al*., 2009). They have great natural diversity and generally are made up hydrolysable and condensed tannins (Okwu and Njoku, 2009). They have pain killing properties. Tannic acids are used for prevention of loss of plasma and limiting secondary infection in burn wounds. Tannins-rich plants extracts are used by Asian natives for the treatment of ulcers. Tannins have uniquely high affinity for precipitating proteins and complexing with all kinds of biomolecules (Peru, 2001; Dharmananda, 2007). Phenols or carbolic acids are aromatic alcohols consisting of a benzene ring bonded directly to a hydroxyl group (OH) (De-Reuter, 2005). They are weakly acidic, and have long history of roles in antisepsis and disinfection (Okigbo *et al*., 2009). They are used as the starting ingredients in the industrial production of drugs, herbicides. synthetic resins and additives to inhibit microbial growth in various ranges of pesicides (Greener Industry, 2009). Phenolics slow growth, block microbial cell division and enzyme activity. According to Okwu *et al*. (2007) they caused swelling of fungal hyphae tips, plasma seeping and leaking around hyphae tips, cell wall distortions, abnormal branching or fusion of hyphae surface.

Saponins are glycosides of both triterpenes and steroids known for the soap-like foaming they produce in aqueous solutions. Saponins have a characteristic property of being bitter or astringent (Okigbo *et al*., 2009). Their soap-like nature makes them useable as surfactants and adjuvants for vaccines to enhance macromolecule penetration (Wikipedia, 2008). Saponins can ward off microbes and this makes them good candidates for treating yeast, viral and fungal infections. They are known to play a role in cytolysisby complexing with cell membrane bilayers (Okwu and Njoku, 2009) sometimes creating pores on them (Rongai *et al*., 2012).

In recent years noted a study from Italy, plants of the family *Brassicacaea* have attracted scientific attention due to their high contents of glucosinolates. These glycosidic compounds have no biocidal activity in their native forms but are converted through enzymatic hydrolysis within living systems to their active forms (isothiocyanates) which have strong cytotoxic activity (Rongai *et al*., 2012). Methyl isothiocyanate (MITC) and benzyl isothiocyanate (BITC) were reported present in *Carica papaya* (Morton, 987; Cornell University, 2012). BITC occurs in the range of 1.37-1.96% in the *Family Caricaceae* (Tang et al., 2001). According to Reulas *et al.* (2003) BITC isolated from Papaya seeds is known to be a strong antifungal compound. At 0.5mg/ml the compoundinhibited the fungus *Alternaria alternata* causing post-harvest rot of tomato. Later evaluations indicated that aqueous seed extract from *C. papaya* was superior to metalaxyl (ridomil) in inhibition effects against *Phytophtora palmivora* inciting black pod disease of cocoa*in vitro*. Investigators believed BITC was responsible for this reported activity(Wokocha and Nwaogu, 2008; Enyiukwu and Awurum, 2013).Furthemore, for instance, the

efficacy of *Afromonium meleguata* and *Monodora myristica*seeds and leaves extract against *Sclerotium rolfsii*, the incitant of basal stem rot of cowpea, according to Okwu and Njoku (2009)were due to their high contents of alkaloids. Enyiukwu and Awurum (2011; 2012), also attributed the potency of *Piper guineense* seed extracts in the inhibition of spore germination, mycelial elongation, development and spread of the causal organism of anthracnose (*Colletotrichum destructivum*) of cowpea in culture and glasshouse, largely to its high contents of alkaloids and perhaps, terpenoids in the test seeds extracts. Similarly, alkaloids are also reported responsible for the fungitoxic activities of extracts of *Raecimus communis* and *Datura stramonium* against several phytopathogenic fungi, while *Cucumina longa* and *Azadirachta indica* contain bioactive terpenoids which have been implicated for activities against a broad array of plant pathogenic fungi and bacteria (Gurja *et al.,* 2012).Amadioha and Obi (1998) in a study with spices suspected and attributed the potency of extracts of *Xylopia aethiopica* againt *C. lindemuthianium* the incitant of antheacnose of cowpea to perhaps the presence of ***xylopic acids*** in the plant materials.

Functional chemical groups

In a study on cocoyam in Nigeria, it was inferred that rot causing fungi produce pectinolytic and cellulolytic enzymes which degrade cell wall polymers and as such make available carbon sources for the invading pathogens. *Ocimum basilium* extract which inhibited the rot-causing fungi in the study was seen as possessing active principle(s) that arrested the ravages of the pathogens or their enzymes or both (Ugwuoke *et al*., 2008).Tests with infra-red reveal that these phytochemicals, according to several workers, contain many chemically potent functional groups. Functional groups are specific groups of atoms or bonds within molecules that are responsible for the characteristic chemical reactions of the molecules. Same functional groups are known to undergo same or similar chemical reaction regardless of the size of the molecule it is a part of. However its relative reactivity is modified by nearby functional groups. Functional groups include amides, carbonyls, esters, aldehydes, phenyls, hydroxyls, ethyls, methyls etc. With these functional groups extracts (isolates) establish bonds with target enzymes, hormones, organelles or processes of pathogens to their harm (Brown, 2006; Okwu and Ukanwa, 2010).Echeverrigaray *et al* (2010) reported that hydroxyl and carbonyl groups or substituents on carbon skeletons of various monoterpenes are responsible for their inhibitory activity against soil inhabiting nematodes. The report further suggested that in addition, the position of these functional groups also influence the observed activity. Malheiros *et al*. (2005) in agreement from a parallel study, reported that dramane sesquiterpenes from*Drimys brasilliensis* inhibited a variety of human myco-pathogens including the stubborn *Epidermophyton floccosum*; and showed that their activity decreased 8 times (from 3 mg/ml to 25 mg/ml) when a bulky substituent (*p*- methoxy or *p*-hydroxycinnamoyl) is

present in carbon position 1. Some of the essential functional groups of a phytochemical are listed in Table 3.

Table 3: Infra-red analysis of isolates from *Afromonium meleguata* and *Monodora myristica* for functional groups.

Plant Isolate	Frequency	Functional group	Compound type	Reference
***Afromoniun meleguata* seed**	3413.07	OH	Hydroxyl phenol	Okwu and Njoku, 2009.
	3108.08	C-N	Amine	
	2925.88	C-H	Aliphatic stretching	
	2854.04	C-H	Aliphatic stretching	
	1709.15	C=O	Carbonyl ester	
	1654.99	C=O	Carbonyl ketone	
	1604.88	C=C	Aromatic	
	1121.00	C-O	Ether	
	3418.90	O-H	Hydroxyl phenol	
	2924.00	C-H	Aliphatic stretching	
	2852.41	C-H	Aliphatic stretching	
	1738.83	C=O	Carbonyl ester	
	1615.89	C=C	Aromatic	
	1455.75	C=C	Aromatic	

	1376.98	C=C	Aromatic substitution
	1164.45	C-O	Aromatic substitution
Afromonium meleguata leaf	3401	O-H	Hydroxyl phenol
	3008	C-N	Amine
	2926	C-H	Aliphatic stretching
	2864	C-H	Aliphatic stretching
	1464	C=C	Aromatic
	1378	C-O	Ester
	1244	C-O	Ether
	1177	C-N	Amine
	1098	C-O	Ether
	1050	=C-H	Aromatic substitution
	801	=C-H	Aromatic substitution
	723	=C-H	Aromatic substitution
*Monodoramyristica*seed	3442.25	O-H	Hydroxyl phenol
	2359.42	C-H	Aliphatic hydrocarbon

	1634.29	C-=O	Carbonyl
	1539.26	C=C	Aromatic
	1455.90	C=C	Aromatic
	668.13	=C-H	Aromatic substitution
Monodora myristica leaf	3421.22	O-H	Hydroxyl
	2854.11	C-H	Aliphatic stretching
	2360.52	C-H	Aliphatic stretching
	1735.85	C=O	Carbonyl ester
	1653.09	C=O	Carbonyl ester
	1558.43	C=O	Carbonyl ketone
	1507.08	C=O	Carbonyl ketone
	1457.81	C=O	Carbonyl ketone
	1375.28	C-O	Ether flavonoid
	1260.39	C-O	Ether flavonoid
	1022.31	C-O	Ether flavonoid
	799.60	=CH	Aromatic substitution

According to Okwu and Uchenna (2009), many other functional groups exist in these phytochemicals besides the ones shown above in Table 3, such as pyrones,double and triple bonds etc. These chemically reactive groups have been postulated to be isolates'

arsenals of attack against metabolic sites and enzymes of pathogens (Okwu and Ukanwa, 2010) and even host plants in the case of phytotoxic chemicals (Echeverrigaray *et al*., 2010).

Characterizedisolates of some extracts

The effectiveness of the activity of phytochemicals against pathogenic organisms has been reportedly suggested to depend on the type and concentration of bioactive principles they contain (Owolade and Osinkalu, 1999). In India, Stripathi and Poongothai (2010) reported that from bioassay-guided fractionation of *Pisonia glandis* a topical medicinal plant, isolated fraction A inhibited the fungus *Monascus purpureus* better than clotrimazole a standard drug against its infections in humans.Of all the plant materials listed in Table 1 above, reportedly used in many researches on toxicity to phyto-parasites, only neem (*Azadirachta indica*) has been most extensively studied, characterized; and asserted to contain the terpenoid *azadirachtin.* Scott *et al*., (2002; 2005) also documented that *Piper guineense* contain the alkaloids *piperine and piperidine* which have strong fungitoxic and pesticidal attributes. Allicin isolatedfrom *Allium spp*. effectively controlled seed-borne *Alternaria spp*. in carrot, *Phytophtora* leaf blight of tomato and tuber blight of potato. Guie *et al.* (2003) indicated the isolation of flavanolglycoside isorhamnetin from*Alchornea cordifolia.* Later investigation revealed the presence of anthocyanidine glycoside in *Alchornea cordifolia*, andfingered this compound to underpin its antibacterial, antiviral and antimicrobial properties (Okwu and Ukanwa, 2010). Polygodial (sesquiterpenes) has been isolated from *Drimys brasilliensis* (Malheiros, 2005). The compound has been found to exhibit insect antifeedant, antimicrobial activities as well as fungicidal activities to yeast, filamentous fungi and the medically difficult to eradicate endomycotic *Epidermophyton fluccosum* (Taniguchi *et al.,* 1998; Lunde and Kubo, 2000; Malheiros*et al*, 2005). Recently a few other plant materials have been examined chemically and their active principles isolated, characterized and documented Table 3.

Table 4: Some characterized plant materials with antimicrobial activities

PLANTS/PLANT MATERIALS	CHARACTERISED ISOLATE(S)	SOURCE
Alchornea cordifolia	*Isopenteneyl guanidine*	Lamikanra *et al*., 1990
Aspilia Africana leaf	*Inisitol*	Ita *et al*., 2010
	Limonene,	

	Alpha-pinene	
	Ethyl 3-(3,4-dihydroxyphenyl) acrylate	Faleye and Ogundaini, 2012
	3-(4-dihydroxyphenyl)-oxo-2H-chromene-6-carbaldehyde	
Ocimum basilicum	*Linalool*	Klimankova, 2008
	Methyl chavicol	
	Eugenol	
	Bergamotene	
	Methyl cinnamate	
	Geranyl acetate	Ozcan and Chalchat, 2002.
	Methyl eugeno	
	Rosmarinic acid	Jamal *et al*., 2002
Stachyterpheta jamaicensis linn vahl	*Lanostance glycoside*	Okwu and Offiong, 2009.
S. jamaicensis	*Steroidal glycoside*	Okwu and Ohenhen, 2010.
Nerium oleander	*Oxoocyl-1-2-hydroxyundecanoate*	Sharma *et al.*, 2010.
	Heptacosane-3enyl-5-hydroxyhexanoate	
	Butelin	
	Butelinic acid	
	Stigmasterol	

		Gupta andMittal, 2010
	Neridienone A	
	Neridienone B	
Syzygium aromaticum	*4-allyl-2-methoxyphenol*	Rahimi *et al*., 2012
Eugenia carophyllata	*Eugenol*	Singh *et al*., 2012
Hyptis suaveolus	*Stigmasterol*	Jasani *et al*., 2012.
	Beta-myrcene	Vijay *et al,* 2011
	Sabenene	
	(2E)-1-(2-hydroxy pheyl)penta-2-en-1-one	Jayakumar and Ganesh, 2012
	1-{(3-hydroxy-5, 5-dimethylcyclohex-3-en-1yl)oxy}hexan-3-one	
Datarium senegalense gmelin	*Tetrahydroxyl anthonyanides*	Okwu and Uchegbu, 2009.
Dataru metel linn	*Alkaloid sitosterol 1*	Okwu and Igara, 2009.
Vernonia amygdalina	*Stigmasterol*	Luo *et al*., 2008
	Chondrillasterol	
	n-Hexadecanoic acid	
	Beta-Sitosterol	Onuegbu, 1996
Melanthera scandens	*Beta-caryophyllene*	Affia *et al*., 2011
	Limonene	

	Beta-phellandrene	
	Alpha-bisabolol	
	Alpha-humulene	
Afromonium meleguata	*Monoterpene indole alcohol*	Okwu and Ojukwu, 2010.
Bridelia ferruginea berth	*Flavonoid chalcones,*	Okwu and Ukanwa, 2010a
	Anthocyanidines	
Garcinia kola seed	*Flavone glycoside*	Okwu, 2007.
Datura metel linn	*Beta-Carboline alkaloid*	Okwu and Igara, 2011.
Ficus exaasperta	*Ficusamide*	Dongfack *et al*., 2012
	Furanocoumarines	
	Bergapten	
	Alpha-terpineol	Oladosu, *et al*., 2009.
	Alpha-pinene	
Peperomia pellucida	*Phytol*	Wei *et al*., 2011
	Hexadeconoic acid	
	Naphthaleno	
	Octadecanoic acidl	
Bryophyllum pinnetum	*Flavonoid glycoside,*	Okwu and Uchenna, 2009.
	Rutin,	
	Kaemoforol-3-glycoside,	
	Beta-Sitosterol	

Cleredendon splendens	*Flavonone-diglucoside, Hispudilin*	Okwu and Uchenna, 2009.
Ageratum conyzoides	*Coumarin*	Wildodo *et al.,* 2008
Cassia alata	*Chrysanthemic acid, Luteoline*	Okwu and Uchenna, 2009.
Nauclea latifolia	*Nauclechine*	Okwu and uchenna, 2009.
Azadirachta indica (Neem)	*Azadirachtin*	Brown, 2006
Alchornea cordifolia	*Anthocyanidine glycosides*	Okwu and Ukanwa, 2010b
Piper guineense	*Piperine, Piperidine.*	Scott *et al., 2002.*
Carica papaya	*Carpaine, Pseudo-carpaine,*	Njoku and Obi, 2009.
Carica papaya	*Xanthine, Violaxanthine*	Njoku and obi, 2009.
Allium cepa L.	*Allicin*	Gurjar *et al.,* 2012.
Allium satvum L.	*Allicin*	Gurjar *et al.,* 2012.
Thymus vulgaris L.	*Caffeic acid*	" "
Ricinus cumminis L.	*Ricinine, Ricininoleic acid*	" "

Plants contain a marvelous array of potent and bioactive chemical compounds which play roles in warding off microbial, pest and herbivoral attacks. A source noted that 12, 000 of such chemical compounds have been isolated from the plant kingdom. In the thoughts of Pallat (2010), these are still infinitesimal compared to the enormous chemical space provided by the 250,000 species constituting the world's flora. Identification, isolation, characterization and purification of novel potent compounds of plant origin will pave way to sustainable food production; reduceeco-contamination while delaying or reversing pathogen resistance to plant health management chemicals.

Mode of action of isolates from biopesticides

Mechanism of action (MOA) of a chemical substance refers to the specific biochemical interaction through which a chemical substance exerts or produces its effects. MOA includes a crystal clear mention of specific molecular targets to which the chemical binds such as enzymes or receptors. For example, in a related field of pharmacology, it is known that salicylic acid, acts by irreversible inhibition of the enzyme *cyclo-oxygenase* and suppressing the production of the hormones **prostaglandins** and **thromboxanes** leading to pain relief. The demand for bio-pesticides especially botanicals however, are seriously constrained by dearth of knowledge of their modes of action, in particular their target specificity, and slow action. These have put them in a serious disadvantage *vis-a-viz* the synthetic chemicals noted for their fast action. Despite the glorious potentials of plant extracts for crop disease management, they are also demerited in being photo and themo unstable, and like their predecessors the *pyrethrins* have short half-life, shelf storageability and are rapidly degraded by ultra-violent radiations (Kumar, 1986; Eno, 2011; Gurjar *et al*., 2012). Aside of the pyrethrins (sodium channel modulators), nicotine (acetylcholine agonist) and rotenone (electron transport inhibitor at *cytochrome A*) whose modes of action have long been determined; of all the plant materials above, only azadirachtin isolatedfrom neem has been clearly reported to act by antagonizing *prothoracicotropichormone* (PTTH) of target organisms (Brown, 2006).From available literature, many reports only offer postulations on the modes of action of these bioactive metabolites. For example, in a parallel study, Okwu and Ukanwa (2010) suggested that anthocyanidine glycoside from *Alchornea cordifolia* inhibited *Klesbiella sp* and *Staphyllococcus aureaus* probably by the mechanism of membrane disruption or enzymes inactivation. Binding to adhesins, proteins, substrate deprivation, and intercalation into cell walls and DNA have been advanced as well by many workers as possible mechanisms for the observed inhibitions (Echeverrigaray *et al.,* 2010; Gurjar *et al.,* 2012).Allicin (Alliaceae) is thought to be readily cell-membrane permeable and undergoes thiodisulphide exchange reaction with amino acids and proteins and its fungitoxic property is assumed pivoted on this attribute (Shusarenko *et al.,* 2008). Besides it is also thought that the mode of action of allicin may also be by mediation of lipoperoxide production in fungal plasmamembrane leading to increased permeability (Rongai et al., 2012). With regard to saponins, remarked the fore-going source, their mechanism of antifungal action is not well understood but it is believed to complex with sterols in the cell membrane leading to pores formations and consequent loss of cell membrane integrity.Furthermore, according to Brown (2006), cinnamaldehyde (Cinnacure, Cinnamite) a botanical pesticide is believed to impair energy production of target organisms. He however reported that its exact mode of action is not well understood though interference with glucose uptake is assumed. Concrete studies to ascertain these claims and postulations seem lacking. However, experts maintain that unless something is done drastically to improve the effectiveness of biopesticides, the growth in their popularity will remain only gradual (Biopesticides,

2012). We, as a matter of necessity must understand how these active isolates interfere with the physiology of the target organisms before thinking of improvements. To this end therefore, the challenges facing us in this area of plant disease management now are to determine:

- What constituent(s) make up the test extracts and what functional groups does the extract possess?
- What are their binding sites on the target pathogen?
- What metabolic processes or pathways do they affect by so doing?
- How do theytherefore effect the observed inhibitions and/oreffect kills on the target pathogen?
- In most cases listed in Table 1, **these are notthoroughly understood yet.**

CONCLUSION

Reports show that majority of the evidences of plant-based chemical activity against plant pathogenic fungi and other micro-organisms of agricultural importance were provided from *in vitro* evaluations. Crude extract evaluations have become very rudimentary. Numerous phyto-chemicals which have demonstrated *in vitro* effects should be evaluated on-farm under natural or induced infections, to further determine and establish their efficacy in controlling the incidences of pathogen-borne diseases in crops in a multi-factor environment. Information on the active ingredients of plant extracts is sparingly available while that ofthe mechanism of action of isolates is almost non-existent.These are high profile, high-tech areas of scientific endeavours, and multi-disciplinary in nature.Therefore further focused and articulated researches to improve the effectiveness, target specificity and shelf storageability of botanicals are pressingly imperative. To achieve this, we must liaise and collaborate effectively with scientists in biochemistry, plant physiology, biotechnology, pharmacology and natural products chemistry. It is in the light of this kind of collaboration and purpose-guided assays that the much anticipated outcome of identifying, isolating, characterizing and understanding isolate-pathogen interaction which will lead us to the knowledge of the mechanisms of action (MOA) of the active principles of plant-based natural products will be achieved in the near future.

SUGGESTIONS AND FUTURE TRENDS

1. Investigators should be encouraged to conduct on-farm evaluations of crude plant extracts against a wide range of pathogens of various high value field and vegetable crops. *In vitro* trials have become too narrow for us to base our conclusion of bioefficacy of crude extracts against pathogenic fungi upon.

2. Given proven phyto-fungal toxicity of the plant materials and assertions on their effectiveness especially from actual field trials in the management of plant health challenges;many concerted and directed efforts and thrusts should hence be geared

toward chemical examinations of the plant materialsin all future investigations with a view to:

- Determining their phytochemical compositions,
- Determining their chemical functional groups and their relative positions on the carbon skeletons,
- Isolating their active ingredients,
- Elucidating and characterizing the structure of isolates so as to enhance:
- Studies on their modes of action on pathogens,
- Phytotoxicity of the principles on host plants and,
- Possible means of improving their effectiveness and synthesis

3. Departmental collaborations should be sought with highly equipped and established laboratories in the USA, UK, China and India to enable investigators overcome complex issues of chemical structure elucidation of isolates and isolate-pathogen interaction.

4. Cost-benefit evaluations should be incorporated in our trials to scientifically establish the cost effectiveness of the plant extracts *vis-a-viz* synthetic chemical product rather than base this on guess works. Appropriate tests on the mammalian toxicity of isolates should thoroughly and speedily conducted to overcome the challenges bans after introduction of products

REFERENCES

Adjaye-Gbewonyo, D., Quaye, E. C. and Wubah, D. A. (2010). The effects of extracts of *Piper guineense*seed on insect pest damage to cowpea. *Journal of young Investigators* 20(1): 264 - 256.

Abawi, G. S. and Hunter, J. E. (1979). White mould of beans: biological control methods. New York's Food and Life Science Bulletin NO 77.

Adebayo, E. A., Ishola, O. R., Taiwo, S. O. Majolagbe, O. N. and Adekeye, B. T. (2009). Evaluation of the methanol extracts of *Ficus exasperata* stem bark, leaf and root for phytochemical analysis and antimicrobial activities. *African Journal of plant Science* 3(12): 283-287.

Adeshina, G. O., Kunle, J. A., Onaolapo, J. A., Ehinmidu, J. O. and Odama, L. E. (2012). Phytochemical and antibacterial studies of the hexane extract of Alchornea cordifolia leaf, Phytochemicals as neutriceutical – Global approaches to their role in nutrition and health. Rao, V. (Ed). Pp 89-96. www.intechopen.com/books/phytochemicals-as-...

Affia, B. F., Tizibo, Z. F., Koffi, A. M., Chalard, P. and Figueredo, G. (2011). Chemicalcomposition of essential oil of *Melanthera scandens* (Schum et Thonn.) Roberty. *World Applied Sciences Journal* 15(7): 992-995.

Agbenin, O. N. and Marley P. S. (2006). In vitro assay of some plant extracts against *Fusarium oxysporium f. sp. lycoperci* causal agent of tomato wilt. *Journal of Plant Protection Research* 46(3): 215-220.

Agrois (2004). Plant Pathology (Online PDF). Academic Press, 1430Pp.

Akinbode, A. O. (2010). Evaluation of antifungal efficacy of some plant extracts on *Curvularia lunata,* the causal organism of maize leaf spot. *African Journal of Environmental Science and Technology* 4(11): 797-800.

Aliyu, A. B., Musa, A. M., oshanimi, J. A., Ibrahim, H. A. and Oyewale, A. O. (2008). Phytochemical analysis and mineral composition of some medicinal plants of Northern Nigeria. *Nigerian journal of PharmaceuticalSciences* 7(1): 119-125.

Amadioha, A. C. (2003). Evaluation of some plant leaf extracts against *Colletotrichum lindemuthianum* in cowpea. *Acta Phytopathologica et Entomologica Hungarica* 38: 259 – 265.

Amadioha, A. C. (2001). Fungicidal activity of some plant extracts against *Rhizoctonia solani* in cowpea. *Archives of Pythopathology* 33: 509 – 517

Amadioha, A. C. (2002). Fungitoxic effects of *Azadiractha indica* against *Cochliobolus myabeanus* causing brown spot disease of rice. *Arch. Phytopathologica Pflanz* 35: 37- 42.

Amadioha, A. C. (2003). Evaluation of some plant extracts against *Colletotrichum lindemuthianum* in cowpea. *ActaPhytopathologica et Entomologica Hungarica* 38(3-4): 259 – 265.

Amadioha, A. C. (2004). Control of black rot of potato caused by *Rhizoctonia bataticola* using some plant leaf extracts. *Archives of plant Pathology and Plant Protection* 37: 111 – 117.

Amadioha, A. C. (2012). Reducing food losses through sustainable method of plant disease management: an imperative for actualization offood security. A paper presented at the 13th inaugural lecture MOUAU, June, 2012.

Amadioha, A. C. and Obi, V. I. (1998). Fungitoxic activity of extracts from *Azadirachta indica* and *Xylopia aethiopica* on *Colletothrichum lindemuthianum* in cowpea. *J. Herbs,SpicesMedicinal Plants* 6(2): 33 – 40.

Ambang, Z., Ndongo, B., Essono, G., Ngoh, J. P., Kosma, P., Chewachong, G. M. and Asanga, A. (2011). Control of leaf spot disease caused by *Cercospora sp*. on groundnut (*Arachis hypogaea*) using methanolic extracts of Yellow oleander (Thervetia peruviana). Seeds. *Australian Journal of Crop Science* 5(3): 227-232.

Amos-Tautua, B. M. W.,Angaye, S. S. and Jonathan, G. (2011). Phytochemical screening and antibacterial activity of methanol and chloroform extracts of *Alchornea cordifolia*. *Journal of emerging Trend in Engineering and AppliedSciences* 2(3): 445-447.

Amienyo, C. A. and Ataga, A. E. (2007). Use of indigenous plant extracts for the protection of mechanically injured sweet potato (*Ipomea batatas* (L.)Lam) tubers. *Scientific Research and Essays* 2(5): 167-170.

Anon. (2012): Key concepts or tools for thinking about Integrated Pest Management (IPM). 13Pp.

Anukworji, C. A., Putheti, R. R. and Okibgo, R. N. (2012). Isolation of fungi causing rot of cocoyam (*Colocasia esculenta* (L.) Schott) and control with plant extracts: (*Allium sativum* L., *Garcinia kola, Azadiachta indica* and *Carica papaya* L.). *Global Advances Research Journal of Agricultural Sciences* 1(2): 33-47.

Asawalam, E. F. and Adesanya, S. O. (2001). Comparison of nematicidal potential of *Azadirachta indica* on the growth and yield of nematode infested okra. *J. sust. Agric. Environ*. 3(2): 85 – 90.

Awurum, A. N. and Ogbonna, M. J. (2013). Field trial on the efficacy of some plant extracts on the control of wet rot of *Amaranthus cruentus* L. induced by *Choanephora cucurbitarium. Continental Journal of Agronomy* 7(1): 10-17.

Awurum, A. N. and Uwajimgba, J. (2013). Varietal screening and comparative toxicity of some plant extracts for control of *Fusarium* wilt of groundnut (*Arachis hypogaea* L.). *Continental Journal of Agricultural Sciences* 7(1): 11-16

Awurum, A. N., Emechebe,, A. N. and Amadioha, A. C. (2001). Effect of planting date on the incidence and severity of some fungi diseases of cowpea in the humid tropics of Southeast Nigeria. *J. Sust Agric. Environ* 3(1): 31 – 38.

Awurum, A. N., Okwu, D. E. and Onyekoro, P. O. (2005). *In vitro* and *in vivo* control of *Colletotrichum destructivum* in cowpea using some plant extracts. *J. Sci., Tech and Envt*. 5(1-2): 52 – 57.

Bennett, D. (2005). Farm Press – New study shows the benefits of fungicides use. www.westernfarmpress.com/new=study-shows... Retrieved Oct. 12, 2012.
Babu, J. Muzafar, A. D. and Vinod, K. (2008). Bioefficacy of plant extracts to control *Fusarium solani f. sp. melongenae* incitant of brinjal wilt. *Global Journal of Biotecnology and Biochemistry* 3(2): 56-59.

Brown, A. E. (2006). Mode of action of insecticides and other related pest control chemicals for production agriculture, ornamentals and turf. Pesticide education and assessment program.Retrieved May 30, 2012. *http://pesticide.umd.edu*

Biobank (2009). Farming methods comparison. www.omz.jp/?page_id=271 Retrieved May 9. 2010.

Biopesticides (2012). Merits and Demerits of Different Kinds of Biopesticides. (Online Edition). 13Pp. Retrieved from: www.biopesticides.com/merits_and_demerits...hml November, 2012.

Chuku, E. C., Osakwe, J. A. and Eke, J. (2010). Fungi flora of melon seeds (*Citrullus colocynthis*) and their response seed treatment. *Scientia Africana* 9(2): 77-82.

Croplife (2012). Value of fungicides in US crop production.
www.croplifefoundation.org/upload/o... Retrieved Oct. 12, 2012.

Choi, G. J., Jang, K. S., Kim, J. S., Lee, S. W., Cho, K. Y., Cho, K. Y. and Kim, J. C. (2004). *In vivo* fungal activity of 57 plant extracts against six plant pathogenic fungi. *Plant Pathology Journal* 20: 184 – 191.

Classwebs (2012). Rachael Carson's Silent Springand beginning of the environment movement in the USA. www.classwebs.spea.indiana.edu/baker/v600/rachael_carson November, 2012.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12: 564 – 582.

Cornell University (2012). Medicinal plants for livestock – *Caricapapaya* – chemical compounds, toxicity, uses and efficacy. Department of Animal Science, 2012.

Debnath, A. Sultana, A. and Rashid, A. Q. M. (2012). Effect of seed-borne fungi on the germinating seeds and their biocontrol in maize. *Journal of Environmental Science and NaturalResources* 5(1): 117-120.

De Ruiter, J (2005). Alcohol structure and chemistry. In: principles of drug action 1 Spring, 2005
Dharmananda, S (2007). Gallnuts and the uses of tannins in Chinese medicine. Retrieved April 14, 2007,. Http://www.itnonline.org/arts/gallunts.htm

Dongfack, M. D. J., Lallemand, M. C., Kuete, V., Mbazoa, C.D., Wansi, J. D., Trinh-van-Dufat, H., Micheal, M. and Wandi, J. (2012). A new sphingolipid and furanocoumarins with antibacterial activity from *Ficus exasperate*. *Chemical and Pharmaceutical Bulletin* 60(8): 1072-1075.

Earnsworth, N.R. (1990). Bioactive compounds from plants. In: CIBS Foundation Symposium No 154, John Wiley and Sons, 1 - 7.

Echeverrigaray, S., Zacaria, J. and Beltrao, R. (2010). Nematicidal activity of monotepenoids against the rootknot nematode *Meloidogyne incognita*. *American Phytopathology* 100(2): 199 – 203.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2009). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4(7): 685 – 688.

Emechebe, A. M and Shoyinka, S. A. (1985). Fungal anmd bacterial diseases of cowpea in Africa. In: Singh, R.S. and Rachie, K. O. (Eds). Cowpea Research, Production and Utilization. John Wiley UK, 173 – 192.

Emechebe, A. M. and Florini, D. A. (1997). Fungal and bacterial diseases of cowpea. In: Advances in Cowpea Research. Singh, R. S., Morgan-Raj, D. and Jackai, L.E.N (Eds). IITA, 176 – 183.

Enyiukwu, D. N. and Awurum, A. N. (2011). Effects of phytochemicals from *Carica papaya* roots and seeds and *Piper guineense* seeds on the germination of spores of *Colletotrichum destructivum*. *Continental Journal ofBiological Sciences* 4(20): 55 – 59.

Enyiukwu, D. N. and Awurum, A. N. (2012). Comparative fungitoxicity of benomyl and extracts of *Carica papaya* roots and seeds and *Piper guineense* seeds on *Colletotrichum destrucivum* O'Gara. *Continental Journal of BiologicalSciences* 6(1): 38 -4 2

Enyiukwu, D. N. and Awurum, A. N. (2013). Fungitoxic effects of *Carica papaya* and *Piper guineense* extracts against*Colletotrichum destructivum* in the glashouse. *Continental Journal of Agricultural Sciences* 7(1): 23-28.

Enyiukwu, D. N. and Awurum, A. N. (2013). Fungitoxic principles and antifungal activity of extracts from*Carica papaya* and *Piper guineense* on*Colletotrichum destructivum*. *Continental Journalof Biological Sciences* 6(1): 29-36.

Eno, I. P. (2011). Field trial on the potential of Anacardium occidentalis for the control of cowpea anthracnose disease in Nigeria. A MSc Thesis presented to the Department PHM, MOUAU, 2011.

Elloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial compounds from plants? *Journal of Ethnopharmacology* 60: 1-8

Faleye, F J. and Ogundaini, O. A. (2012). Evaluation of antioxidant and antimicrobial activities of two isolates from *Aspilia Africana* (Pers) C. D. Adams. *International Research Journal of Pharmacy* 3(7): 135-138.

Green, R. J. (2004). Antioxidant activity of peanut plant tisssues. A MSc Thesis North Carolina State University.

Greener Industry (2009). Phenols – Uses. www.greenerindustry.org/pages/phenol/2phenoluses.htm Retrieved Sept. 4, 2009.

Guie, Y. C., Jun, Y. L., and Ren, X. T. (2003). Isorhamnetin from *Alchornea cordifolia* plant. *Chinese Chemical Letters* 14(2): 176-180.

Gupta, R., Khokhar, M. K. and Lai, R. (2012). Management of black mould of onion. *Journal of Plant Pathology andMicrobiology* 3(5): 3-5.

Gupta, V. and Mittal, P. (2010). Phytochemical and pharmaceutical potential of *Nerium oleander*: A review. *International Journal of Pharmaceutical Sciences and Research* 1(3): 23-27.

Gurjar, M. S., Ali, S., Akhtar, M. and Singh, K. M. (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences* 3(3): 425 – 433.

Gujar, J. and Talwankar, D. (2012). Antifungal potential of crude plant extracts on some pathogenic fungi. World *Journal of Sciences and Technology* 2(6): 58-62.

Harish, s., Saravankumar, D., Ebenezer, E. G. and Seetharaman, K. (2008). Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. *Biocontrol* 53: 555 – 567.

Harisisaranraj, R., Suresh, K and Saravanababu, S. (2009). Evaluation of chemical composition of *Rauwolfia serpentine* and *Ephedra vulgaris. Advances in Biological Research* 3 (5-6): 174-178.

Ibrahim, T. A., Adetuyi, F. O. and Ajala, L. (2012). Phytochemical Screening and Antibacterial activity of *Sida acuta* and *Euphorbia hirta. Journal of Applied Phytotechnology in Environmental Sanitation* 1(3): 113-119.

Igboh, M. N., Ikewuchi, C. J. and Ikewuchi, C. C. (2009). Chemical profile of *Chromolaena odorantum* L. leaves. *Pakistan Journal of Nutrition* 8(5): 521 – 524.

Ilundu, E. M. (2011). Evaluation of some aqueous plant extracts used in the control of pawpaw fruit (*Carica papaya* L.) rot fungi. *Journal of Applied Biosciences* 37: 2419-2424.

Islam, M. T. and Faraq, A. N. (2012). Effects of some medicinal plants on damping-off disease of winter vegetables. *World journal of Agricultural sciences* I7(11): 1498-1503.

Ita, B. N., Koroma, L. and Kormoh, K. (2010). Isolation and characterization of inositol from the ethanolic leaf extract of *Aspilia Africana. Journal of Chemical and Pharmaceutical Research* 2(2): 1-6.

Jayakumar, S. V. and Ganesh S. M. K. (2012). New enolic type bioactive constituents from *Hyptus suaveolens* (L.) Poit. *Asian Journal of Plant Sciences and Research* 2(4): 403-408.

Jasani, K. P., Bhinani, K. M. Dave, P. M. and Ushir, Y. V. (2012). Isolation and determination of triterpenoid from roots of *Hiptus sauveolens. Pharmtechmedica* 1(2): 81-85.

Jamal, J., Khalighi, A., Kashi, Bais, H. P. and Vivanco, J. M. (2002). Chemical characterization of basil (*Ocimumbasilicum* L.) found in local accessions in traditional medicines in Iran. *Journal of Agricultural and Food Chemistry* 50: 5878-5883.

Jeruto, P., Charles, M., Lukhoba, C. and George, O. (2011). Phytochemical constituents of some medicinal plant used by the Nandis of south Nandi District of Kenya. *Journal of Animal and Plant Sciences* 9(3): 1201-1210.

Klimankova, E., holandova, K., Hajislova, J., Cajka, T., Poustka, J. and Koudela, M. (2008). Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food Chemistry 107: 464-472.

Kumar, R. (1986). Insect Pest Control (*Reference to African Agriculture*) Edward Arnold Press UK, 296 Pp.

Kuberan, T., Balamurugan, A., Vadhyapallavi, R., Nepolean, P., Jayanthi, R., Beulah, T. and Premkumar, R. (2012). *In vitro* evaluation of certain plant extracts against *Glomerella cingulate* causing brown blight of tea. *World Journalof Agricultural Sciences* 8(5): 464-467.

Lamikanra, A.,Ogundani, A. O. and Ogunbamila, F. O. (1990). Antibacterial constituents of *Alchornea cordifolia* leaves. *Phytotherapy Research* 4: 98-100.

Luo, X., Lin, C., Izibigvie, E. B. and Lee, K. S. (x). Chemical analysis of VA (*Vernonia amagdalina*) organic components showing anticancer activities against MCF-7 cells. Seventh International Symposium on Recent Advances in Environmental Health No O-08, p8.

Lunde, C. and Kubo, I. (2000). Effects of polygodial on the mitochondrial ATPase of *Saccharamyces cerevisiae*. *Antimicrobial and AgentsChemotherapy* 44: 1943-1953.

Madden, L. V., Hughes, G. and Van der Bosch (2008). The Study of Plant Disease Epidemiology. Minnesota, USA- APS, 421Pp.

Mitali, M. P., Manoranjan, K. and Sahu, R. K. (2012). Bioefficacy of some plant extracts on the growth parameters and control of diseases in *Lycopersicum esculentum.Asian Journal of Plant Science and Research.* 2(2): 129-142.

Morton, J. (1987). Papaya. In: fruits of Warm Climates. Manure Florida. Pp 336 – 346

Montes-Belmont, R. and Prados-Ligero, A. M. (2006). Influence of plant extracts on Sclerotium cepivorium development. *Plant Pathology Journal* 5(3): 373-377.

Nagis, A., Most, F. B., Shahidul, A. and Alam, M. S. (2006). Inhibitory effects of different plant extracts and cow dung on the conidial germination of *Bipolaris sorokiniana*. *Journal of Biological Sciences* 14: 87-92.

Narian, A. (1970). Control of some fungal disease by antibiotics and crude plant extracts. Volume 37, B. Number 5. Retrieved online June 10, 2013.

Nuefack, J., Torp, J., Leth, V., Lekane-Dongmo, J. B., Fotio, D. and Amvan-Zollo, P. H. (2007). Effects of plant extracts and chemical fungicides in controlling rice see-borne fungus under laboratory and irrigated cropping system in Ndop-Cameroon. *African Crop Science Proceedings* Vol. 8, pp 791-796.

Obongoya, B. O., Wagai, S. O. and odhiambo, G. (2010). Phytotoxic effect of selected crude plant extracts on soil-borne fungi of Common bean. *African Crop Science Journal* 18(1): 15-22.

Owolade, B. F. and Oshikanlu, Y.O.K (1999). Evaluation of some plant extracts for the control of brown blotch disease of cowpea in Southwest Nigeria. *J. Sust. Agric Environ*. 1(2): 198 - 202.

Olufolaji, D. B. (1999). Control of wet rot of Amaranthus sp caused by *Choanephoracurcubitarium* with extracts of *Azadirachta indica. J. Sust. Agric Environ* 1: 183 – 190.

Okigbo, R. N. and Nmeka, I. A. (2005). Control of yam tuber rot with leaf extracts of *Xylopia aethiopica and zingiber officinale. African journal of Biotechnology* 4(8): 804-807.

Okigbo, R. N. and Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Afromonium meleguata*) on Post-harvest yam (*Dioscorea spp*.) rot. *African journal of Biotechnology* 5: 727 – 732.

Okigbo, R. N., Anuagasi, C. L. and Amadi, J. E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plants Research* 3(2): 86-95.

Okibgo, R. N., Putheti , R. and Achusi, C. T. (2009). Post-harvest deterioration of Cassava and its control using extracts of *Azadirachta indica* and *Afromonium meleguata. E-journal of Chemistry* 6(4): 1274-1280.

Okwu, D. E., Awurum, A. N. and Okoronkwo, I. J. (2007). Phytochemical composition and *invitro* activity screening of extracts from *Citrus* plants against *Fusarium oxyspporium* of okra plant. *Pest Technology* 1; 145 – 148.

Okwu, D. E. and Njoku, E. E. (2009). Chemical composition and *in vitro* antifungal activity screening of seed and leaf extracts from *Afromonium meleguata* and *Monodora myristica* against *Sclerotium rolfsii* of cowpea. *Pest Technogloy* 3(1): 58 – 67.

Okwu, D. E. and NJoku, E. E. (2010). Monoterpene indole alkaloid from *Afromonium meleguata. Int'l J. Pharmtech Research.* 2(1): 910 – 913.

Okwu, D. E. and Ukanwa, N. (2010b). Isolation, characterization and antibacterial activity screening of anthocyanidine glycoside from *Alchornea cordifolia* leaves. *E-journal of Chemistry* 7(1): 41 – 48.

Okwu, D. E. and Ohenhen, O. S. (2010). Isolation and characterization of lanostane glycoside from leaves of *Stachytarpheta jamaicencis*. *International J. ChemTech Res.* 1(4): 1043 – 1047.

Okwu, D. E. and Ohenhen, O. S. (2010). Isolation and characterization of steroidal glycoside from leaves of *Stachytarpheta jamaicencis*. *Der Chemica Sinica* 1(2): 6 – 14.

Okwu, D. E. and Uchegbu, R. (2009). Isolation, characterization and antibacterial screening of methoxyamine tetrahydroxyanthocyanidines from *Detarium senegalense* stem bark. *African Journal of Pure and Applied Chemistry* 3(1): 001 – 005.

Okwu, D. E. and Uchenna, N. F. (2009). Exotic multifaceted medicinal plants for drugs and pharmaceutical industries. *African Journal of Biotechnology* 8(25): 7271 – 7282.

Okwu, D. E. and Igara, E. C. (2009). Isolation, characterization and antibacterial activity of alkaloid from *Datura metel* leaves. *African Journal of Pharmacy and Pharmacology* 3(5): 277 – 281.

Okwu, D. E. and Igara, E. C. (2011). Isolation, characterization and antibacterial activity screening of alkaloid from *Datura metel leaves. Der Chemica Sinica* 2(2): 261 – 267.

Okwu, D. E. and Iroabuchi, F. (2009). Phytochemical composition and biological activities of *Uvaria chamae* and *Clerodendoron splendens E-journal of Chemistry* 6(2): 553-560.

Okwu, D. E. and Ukanwa, N. (2010a). Isolation and characterization of flavonoids chalcones and anthocyanidines from *Bridelia ferruginea Benth. Der Chemica Sinica* 1(2): 21 – 28.

Okwu, D. E. and Morah, F. N. I. (2007). Isolation and characterization of flavonone glycoside 4', 5,7-trihydroxy flavone rhamnoglucose from *Garcinia kola* seed. *Journal of Applied Sciences* 7(2): 306 – 309.

Oladosu, A. I., Zubair, M. F., Ali, M. S. and Olawore, N.O. (2009). Anticandidal activity of volatile compounds from the root bark of Ficus exasperate Vahl-Holl (*Moracea*e). *Jeobp* 12(5): 562-568.

Opara, E. U. and Obana, F. T. (2010). Performance of some plant extracts and pesticides in the control of bacterial spot disease of *Solanium. Medwell Agric. Journal* 5(2): 45 – 49.

Opara, E. U. and Wokocha, R. C. (2008). Efficacy of some plant extracts on the *in vitro* and *invivo* control of *Xanthomonas campestris pv vesicatoria. Medwell Agric. Journal* 3(3): 163 – 170.

Ozcan, M. and Chalchat J. C. (2002). Essential oil composition of *Ocimum basilicum* L. and *Ocimum minimum* L.in Turkey. *Czech. Journal of Food Science* 20(6): 223-228.

Pachkore, G. L., Dhale, D. A. and Dharasurkar, A. N. (2011). Antimicrobial and phytochemical screening of Hiptus sauveolus (L. Poit) lamiaceae. *International Multidisciplinary Research Journal* 1(4): 1-3.

Pallant. A. C. (2010). Bioactivity of alkaloidal fraction of Tabernaemontana elegans Stapf. Thesis (*MagisterScientiae*) submitted to the Deparment of Pharmacology, Faculty of Health Sciences, University of Pretoria, 2010.

Peru, S. C (2001). The use of tannic acid in the local treatment of burn wounds: Intriguing old and new perspectives. *Wounds* 13 (14) 144 – 158

Purseglove, J.W. (1976). Tropical crops (Dicolyledons). Longman, London. Pp 719 – 724.
Rahimi, A.A., Ashnagar, A. and Nikoel, Hamideh, N. (2012). Isolation and characterization of *4-allyl-2-methoxyphenol* (eugenol) from clove buds marketed in Tehran city Iran. *InternationalJournalof ChemTech Research* 4(1): 105-108.

Reulas, C. Garcia, H.S and Tiznado – Hermandez, M.E (2005). Effect of benzylisothiocyanate on tomato fruit infection development by *Alternaria alternata*www.interscience.wiley.com/journal

Ragsdale, N. N. (1994). Fungicides – USDA – National Agricultural Impact Assessment Programmes. In: Encyclopedia Agricultural Sciences Vol. 2, Academic Press Inc., USA

Rathod, L. R. and Pawar, P. V. (2012). Antimicrobial activity of medicinal plant to control seed-borne pathogen of soybean. *Current Botany*. 3(2): 10-12.

Rongai, D., Milano, F. and Scio, E. (2012). Inhibitory effct of plant extracts on conidial germination of the phytopathogenic fungus *Fusarium oxysporium*. *American Journal of Plant Science* 3: 1693-1698.

Scott, M. I., Puniani, E., Jensen, H., Livesay, J. F., Poveda, L., Sanchas-Vindas, P., Dust, T. and Arnason, J. T. (2005). Analysis of Peraceae germplasm by HPLC and LCMS: A method for isolating and identifying unsaturated amides from *Piper spp. Journal of Agric and Food Chem. http://pubs.acs.org/doi/abs/10.102/jfo48305a*

Sadiku, N. A. and Sadiku, I. B. S. (2011). Indigenous efforts by African farmers in ensuring sustainability in agricultural productivity in the face changing climate: A proceeding of the *Environmental Management Conference*, FUNAAB, Nigeria, 2011.

Sangvikar, V. R. and Wadje, S. S. (2012). *In vivo* testing of plant extracts against seed-borne pathogens. International *Research Journal of Biological Sciences* 1(6): 1-4.

Sarpeleh, A., Sharifi, K. and Somboikar, A. (2009). Evidence of antifungal activity of wild rue (*Peganum harmala L.*) on phytopathogenic fungi. *Journal of Plant Disease and Protection* 116 (5): 208-213.

Senthikumar, N., Murugesan, S., Bhanu, N., Supriya, S. and Rajeshkannan, C. (2011). Biochemical estimation and antibacterial activities of the extracts of*Caesalpinia sappan* Linn. *Bangladesh Journal of Scientific and IndustrialResearch* 46(4): 492-436.

Sharma, P., Gupta, Y. K., Sharma, M. C. and Dobnal, M.P. (2010). Two new compounds from the stem of *Nerium oleander. Indian Journal of Chemistry* 9(B): 374-378.

Singh, J., Baghotia A. and Goel, S. P. (2012). *Eugenia caryophyllata* Thunberg (Family: *Myrtaceae*): A review. *International Journal of Reseach in Pharmacrutical and Biomedical Sciences* 3(4): 1469-1475

Stangarlin, J. R., Kuln, O. J., Assi, L. and Schwan-Estrada, K. R. F. (2011). Control of plant diseases using extracts of some medicinal plants and fungi. In: Science against microbial pathogens; communicating current research and technological advances. Mendez-Vilas, A. (ed). Formatex, 1033-1042.

Soladoye, M. O. and Chukwuma, E. C. (2012). Phytochemical analysis of the stem and root of *Cissus populnea* (*Vitaceae*) – an important medicinal plant in Central Nigeria *Phytologia Balcanica* 18(2): 149-153.

Somda, I., Leth, V. and Sereme, P. (2007). Evaluation of lemongrass, eucalyptus and neem for controlling seed-borne fungi of sorghum grown in Burkina Faso. *World Journal of Agricultural Sciences* 3(2): 218-223.

Suleman, M. N. and Emua, S. A. (2009). Efficacy of four plant extracts in the control of root rot disease of cowpea *AfricanJournal of Biotechnology* 8(16): 3806 – 3808.

Suleman, M. N. and Ogundana, A. F. (2010). Evaluation of two plant leaf extracts on fungi associated with biodegradation of cashew nuts in storage. *Annals of Biological Research* 1(4); 41 – 44.

Suprapta, D. N. and Khalimi, K. (2009). Effects of plant extracts formulation to suppress stem rot disease of vanilla seedlings. *ISSA Journal* 15: 34 – 41.

Sygenta (2012): Researches in crop losses (online). www.sygenta.com/global/corporate/e... Retrieved July, 15, 2012.

Stripathi, S. K. and Poongothai, G. (2010). Bioassaya-guided fractionation and antifungal activity studies on *Pisonia glandis*R. BR. *International Journal of current Research* 10: 35-37.

Taiga, A. (2009). Efficacy of selected plant extracts in the control of fungal dry rot of white yam (*Dioscorea rotundata*) tubers in Kogi State. *American-Eurasian journal of Sust. Agric*. 3: 310 – 313.

Tang, C. S., Syed, M. M and Hamilton R. A. (2001). Benzyl Isothiocynate in the Caricaceae. Phytochemistry (online) 11: 2531-2533.

Tanigushi, M., Yano,Y., Tada, E., Ikenishi, K., Oi, S., Haragushi, H., Hashimoto, K.and Kubo, I. (1998). Mode of action of polygodial an antifungal sesquiterpene dialdehyde. *Agricultural and Biological Chemistry* 52: 1409-1414.

Uchegbu, R. I. and Okwu, D. E. (2012). An evaluation of the phytochemical and nutrient composition of seeds and stem bark of *Detarium senegalense* Gmelin *Journal of Natural Sciences Research* 2(5): 107 – 111.

Ugwuoke, K. I., Onyeke, C. C. and Tsopmbeng, N. G. R. (2008). The efficacy of botanical protectants in the storage of cocoyam (*Colocasia esculenta* (L.) Shott). *Agro-Science-Journal of Tropical Agriculture, Food, Environment and Extention* 7(2): 93-98.

Vijay, R. A. J., Pandiyarajan, V. and Petchimuthu, K. (2011). Comparison of chemical composition of the essential oil of *Hyptus sauveolens* (L.) Poit leaves from different regions of Tamil Nadu.*International Journal of Pharmaceutical Sciences and Research* 2(11):2822-2824.

Wei, L. S., Wee, W., Siong, J. Y F. and Syamsumir, D. F. (2011). Characterization of anticancer, antimicrobial, antioxidant properties and chemical composition of *Pereromia pellucida* leaf extract. *Acta Medica Iranica* 49(10): 671-674.

Wessells, N. K. and Hopson, J. L. (1988). Biology. New York, Random House Press. Pp. 505 – 521

Widodo, G. P., Sukandar, E. Y., Sukranso, and Adnyana, K. I. (2008). A coumarin from *Ageratumconyzoides* L. *International Journal of Pharmacology* 4(1): 56-59.

Wokocha, R. C. and Okereke, V. C. (2005). Fungitoxic activity of extracts of some medicinal plants on *Sclerotium rolfsii* causal organism of basal stem rot of tomato. *Nigeria Journal of Plant Protection* 22: 106 – 111.

Wokocha, R.C and Nwaogu G.A (2008). Comparative toxicity of extracts of three medicinal plants and Ridomil (metaxyl) on *phytophtora palmivora* and varietal resistance of cocoa to the black pod disease in Ikwuano L.G.A, Abia State, Nigeria. *Nigeria Journal of Botany* 21(2): 428 – 436

Yadav, R. N. S. and Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology* 3(12): 10-14.

CHAPTER SEVEN

COMPREHENSION ON USE OF MEDICINAL PLANTS TO CURE DIARRHEA AMONG THE TRIBALS OF MAYURBHANJ DISTRICTS, ODISHA, INDIA

Laxmipriya Padhi and Sujogya Kumar Panda
Department of Zoology; North Orissa University; Baripada, Odisha, India-757003

ABSTRACT

This chapter presents a comprehension on ethnomedicinal uses of medicinal plants by the indigenous tribals of District Mayurbhanj, Odisha, India. In addition to this the chapters also scientifically validated how many of these plants are active to kill bacteria responsible to cause infectious diarrhea. Aqueous and methanol extracts of 150 plants were recorded as medicinal value, of which 136 plants were tested for antibacterial activity using agar well diffusion against eight pathogenic bacteria involved in diarrhea and dysentery. The results indicated that out of 136 plants species, 100 species exhibited antibacterial activity against one or more test organisms. In total 288 (144 methanolic+144 aqueous) extracts, 107 methanolic and 74 aqueous extracts expressed antibacterial properties. From the screening results plant family's viz. Fabaceae (08), Rutaceae (07), Combretaceae (06), Caesalpinaceae (06), Anacardiaceae (05) and Apocynaceae (04) showed highest antibacterial activity in terms of test plant numbers. However, families viz. Burseraceae, Crassulaceae, Hypoxidaceae, Liliaceae, Lygodiaceae, Musaceae, oxalideceae, Pedaliaceae, Strychnaceae, Trapaceae, Ulmaceae did not showed activity against any test organism. Moreover, Euphorbiaceae, Rubiaceae are the two largest families from which plants did not show result significant activity against diarrheal causing bacteria. Among the plants viz. Acacia leucophloea, Adhatoda vasica, Andrographis paniculata, Annona reticulate, Anogeissus latifolia, Anthocephalus chinensis, Bombax ceiba, Buchanania lanzan, Butea monosperma, Careya arborea, Cassia fistula, Centella asiatica, Cissampelos pareira , Coccinia grandis, Croton roxburghii, Curculigo orchioides, Diospyros melanoxylon, Eleutherine bulbosa, Erycibe paniculata, Ficus racemosa, Flemingia nana, Helicteres isora, Holarrhena pubescens, Helicteres isora, Lannea coromandelica, Litsea glutinosa, Mesua ferrea, Mimusops elengi, Moringa oleifera, Nyctanthes arbortristis, Phyllanthus emblica, Piper longum, Pterocarpus marsupium, Pterospermum acerifolium, Punica granatum, Quisqualis indica, Semecarpus anacardium, Smilax zeylanica, Terminalia arjun, Terminalia bellirica, Tinospora cordifolia and Vitex negundo experimentally proved to inhibit most of the diarrheal causing bacteria.

KEYWORDS: *Antidiarhheal, Similipal Biosphere Reserve, Preliminary screening, Plant extracts, Ethnomedicinal uses*

INTRODUCTION

Diarrhea, particularly infectious diarrhea is the second leading cause of mortality and morbidity throughout the world in children less than 5 yrs of age. This is especially true in developing countries like India where there is poor sanitation and overcrowding.

Estimation of crude death rate due to diarrhea in India was 9.3 per 1000 population (WHO, 2005). If this continues, then the predicted case of burden will rise to 126.35 cores during 2016 (WHO, 2005). Among the leading causes of infectious diarrhea, *Salmonella* and *Shigella* contributes highest number. The current the chemotherapeutical treatment of Salmonellosis and Shigellosis is complicated as a result of drug resistance. Moreover, majority of the people in these developing countries have no access for modern health care facilities. This necessitated the search for alternative therapies such as, the use of medicinal plants. Shigellosis is an important cause of diarrheal deaths. It has been reported that not less than 140 million cases of Shigellosis occur worldwide with 6,00,000 deaths annually, 60% of such deaths are of under 5 years old children (Peirano et al., 2006; Sur et al., 2004). Among the different species of *Shigella, S. dysentriae* is known for its fatality, and life threatening situation. The emergence of multiple drug resistant strains of diarrheagenic pathogens has made the treatment of dysentery more difficult hence there is increasing interest in plants as source of antimicrobial agents for the treatment of such diseases (Munshi et al., 1987; Monroe and Polk, 2000). Salmonellosis, another type of diarrheal diseases is caused by a group of bacteria called *Salmonella*. It is primarily transmitted through ingestion of contaminated food by infected faeces from man or animal, through fecal oral route. Active cases of *Salmonella* in man are source of contamination and transmission to other human beings and to lower animals. Strains of *Salmonella* species with resistance to antimicrobial drugs are now widespread in both developed and developing countries. In developed countries, it is now increasingly accepted that for the most part such strains are zoonotic in origin and acquire their resistance in the food-animal host before onward transmission to humans through the food chain. On the other hand, indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases lead to high resistance among the strains. This has forced researchers to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. Several studies have identified that the compounds within herbal plants are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al. 2004); some traditional remedies have already been produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004).

Diarrheal disease continues to be a major cause of morbidity and mortality throughout the world, particularly among children in developing countries, often as a result of infection by bacteria, viruses, and protozoal parasites. Given the increasing resistance in many common pathogens to currently used chemotherapeutic agents, there is renewed interest in the discovery of novel compounds that can be used to fight infectious diseases. There have been numerous studies that have served to validate the traditional use of medicinal plants used to treat or prevent diarrhea. Several methods can be used

for selecting plants of potential therapeutic interest (Vlietinck and Vanden-Berghe, 1991; Farnsworth, 1993). The search can follow three main routes: random, ethno (including ethnobotanical, ethnomedical and ethnopharmacological) and ecological search (Fabricant and Farnsworth, 2001). The ethnobotanical and ethnopharmacological approach uses information obtained from traditional medical practitioners and other people such as village elders and local women who are traditional users of medicinal plants. Ethnomedicinal reports are available on the use of plants for the treatment of diarrhea and dysentery by the tribals from district Mayurbhanj (Rout and Pandey, 2002; Pandey and Rout, 2002; 2006; Rout and Panda 2010, Panda et al., 2011a,b,c; Panda et al., 2012; Panda, 2014). There is limited information on the safety of traditional plant extracts, although some clinical trials have evaluated the safety and tolerability of herbal medicine preparations used to treat diarrhea and generally indicate that minimal side effects are observed. Based on earlier reports and data collected from the present ethnomedicinal uses, plants were selected to find out the experimental validation of tribal knowledge for the curing of infectious diarrhea.

LITERATURE REVIEW

Diarrhea poses a significant economic and societal burden throughout the world both in developing and developed countries. Annually, more than five million people, 80% of whom are less than one year of age, die from acute infectious diarrhea (Sood and Pacheco, 2002). Additional agents of infectious diarrhea for which clinical diagnostic testing is not routinely available include enterotoxigenic, enteropathogenic, enteroaggregative, and enteroinvasive strains of *E. coli,* toxin-producing *Clostridium perfringens, Staphylococcus aureus, Bacillus cereus* and rotaviruses (Guerrant, 1998).The emergence of multiple drug resistant strains of diarrheagenic pathogens has made the treatment of dysentery more difficult. In developing countries, the majority of people living in rural areas almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea. A range of medicinal plants with anti-diarrheal properties have been widely used by the tribes of Mayurbhanj. The effectiveness of many of these plants has not been scientifically evaluated.

Diarrhea: Definition and related terms

Diarrhea is loosely defined as an alteration in the normal bowel movement characterized by an increase in the volume, frequency and water content of stool (Baldi et al., 2009). The patho-physiology of diarrhea include microbial and parasitic infections (Hodges and Gill, 2010), stress (oxidative or physical) (Soderholm and Perdue, 2006), dysfunctional immunity (Schulzke et al., 2009), disrupt GIT integrity and neurohumoral mechanisms (Vitali et al., 2006; Spiller, 2004) . Diarrhea can also be a symptom of other diseases such as cholera, irritable bowel syndrome (IBS), gastroenteritis (intestinal inflammation and ulcerative colitis) (Schiller, 1999; Baldi et al., 2009), malaria (Gale et al., 2007) and diabetes mellitus (Forgacs and Patel, 2011) etc.

Classification

Diarrheal disease can be either infectious or non-infectious in nature. In infectious diarrhea, the potential causative pathogens include bacterial agents (Mathabe et al., 2006), rarely fungal (Robert et al., 2001), viral and parasite pathogens (Brijesh et al., 2006). Non-infectious diarrhea can be caused by adverse reactions to drugs, toxins, allergy to food, poisons and acute inflammation which promote the release of secretagogues and some enteric nervous system (ENS) receptors (prostaglandin, serotonin, substance P, vasoactive intestinal peptides, and hormone) in the GIT (Wynn and Fougere, 2007) . Diarrhea can be classified in several ways viz. according to the duration of the symptoms (Acute, persistant, chronic); according to stool characteristics or pathological mechanisms (watery, osmotic, altered motility or inflammatory diarrhea) (Ravikumara, 2008); according to age group (infantile, weanling, childhood, adult); based on epidemiological pattern (sporadic, endemic, epidemic, pandemic); based on seasonal (summer, winter, monsoon) etc.

Plant metabolites as potential therapeutic agents

Since ancient time, plants have been used as medicines by peoples. Today, the majority of people in the world use traditional medicines for their primary course of treatment because (i) biomedical healthcare systems and pharmaceuticals are not available in most places, (ii) Synthetic drugs are not safe. Thus, to improve health and to instill pride in traditional knowledge systems, several governments (e.g., China, India, and South Africa) are incorporating traditional healthcare practices into their national regimes (WHO, 2003). Medicinal plants have therapeutic properties due to biosynthesis of various complex phytochemical substances grouped broadly as phenolic compounds, quinones, stilbinoids, flavonoids, tannins, coumaranis, alkaloids, terpenoids, lectins and polypeptides. Pharmacological and clinical studies of phytochemicals in plants have shown that they exhibit various medicinal uses (Van Wyk and Wink, 2004). Synergistic interaction among the multiple phytochemicals may be responsible for the overall bioactivity of a given medicinal plant. Several plant extracts, formulations, or pure natural compounds are used in controlling diverse diseases incluiding diarrhea to parasitic infection in both human and veterinary medicine.

Plant-based remedies in improving diarrhea

Ethnobotanical theories support the effectiveness of plants for therapeutics, incliding all type of gastrointestinal diseases. All animals including humans have develop senses to select appropriate plants for ingestion, digestive enzymes to acquire nutrients from plants, and behaviors or detoxifying enzymes to neutralize harmful plant chemicals (Johns, 1996). Due to the widespread occurrence of diarrhea as a disease together with the prevalence coinciding with human development, plants have featured widely in the management of the disease both in human and veterinary medicines. Johns (1999) postulates that "fundamental forms of medicine involve the gastrointestinal tract"

because of the immediate cognitive link between ingesting effective medicinal plants and relief of gastrointestinal ailments. This cognizant association could be one reason why all known pharmacopeias of the world possess remedies for gastrointestinal illness. Another reason is that gastrointestinal ailments are ubiquitous. Therefore, the need for treatments is universal.

Possible mechanisms of action for anti-diarrheal plants

Plants may act in various ways in alleviating diarrhea. It also providing nutrients and generally increasing gastrointestinal health. However, for treatment purpose plants are known as anti-infectious agents due to their secondary metabolites to fight against diarrheal pathogens.

Antimicrobial: Many plant metabolites are known to exhibit some level of toxicity toward microorganisms. Numerous mechanisms of actions have been hypothesized to explain their antimicrobial activity such as microbial enzymes inhibition, deprivation of essential growth substrates, cell membrane disruption (Cowan, 1999) or direct interference with metabolic pathways.

Antiadhesion: Adhesion of some enteric pathogen to the mucosa epithelium of the host cells is the first important step in intestinal infections that may lead to the development of diseases (Ofek and Sharon, 1990). Application of antiadhesives chemotherapy can be effective only against microorganisms that depend on the surface contact with host cells as prerequisite for survival, multiplication and virulence (Lengsfeld et al., 2007).

Antitoxin: Since enteric pathogens may induce diarrhea through the production of toxin (endotoxin or cytotoxin) the neutralization with plant antidiarrheal compounds may beneficial in the management of diarrhea. Activated charcoals processed from plants are also used as toxin binders.

Immunomodulatory: With immune suppression being a pre-disposing, drugs or medicinal plant preparations with immune stimulating activities may help in attenuating many infectious diseases.

Phytochemicals responsible for anti-diarrheal properties

Much plant-based anti-diarrheal research has analyzed the effects of phytochemicals on intestinal tissues (Brijesh, *et al.*, 2006; Grover, *et al.*, 2002; Sagar, *et al.*, 2005; Shaphiullah, *et al.*, 2003; Shilpi, *et al.*, 2006; Teke, *et al.*, 2007; Gutierrez et al., 2007). Using rodent models, extracts are evaluated for antispasmodic effects, gut motility suppression, or water and electrolyte reabsorption (Akindele and Adeyemi, 2006; Mbagwu and Adeyemi, 2008; Sairam, *et al.*, 2003; Thakurta, *et al.*, 2007) with tannins and flavonoids exhibiting promising results for water and electrolyte retention (Palombo, 2006).

Astringent and pectin-rich plants often are used to treat diarrheal disease, as are opiates that slow smooth muscle contractions of the intestines (Lewis and Elvin-Lewis, 2003). However, these remedies that suppress intestinal function block the symptoms of diarrhea and not the causes.

Bacteria, viruses and parasites are the major causes of infectious diarrhea, with bacteria leading highest (2-4 billion cases of infection with 3-5 million deaths per year (Sanchez and Holmgren, 2005). Phytochemicals inhibit the growth and virulence of diarrhea-causing bacteria in numerous ways. When bacteria invade the intestines, they follow similar etiologies. The sequence, known as the five stages of pathogenicity (Mitchell, 1998), includes: 1) adherence to host tissue, 2) invasion or control of host tissues, 3) multiplication in host tissues or with nutrients from host tissues, 4) evasion of host defenses, and 5) damage and spread.

Phytochemicals can inhibit bacterial growth or virulence at any of these five stages of pathogenicity. For example, mucilaginous, astringent and fibrous properties of some plants can mechanically prevent bacterial adhesion to host intestinal cells by direct competition between plant-derived lectins and bacterial membrane glycosides (Coutião Rodriguez *et al.*, 2001; Rabbani *et al.*, 2004).

Agents involved in causing diarrhea

Bacterial causes of diarrhea

Escherichia coli

E. coli is a gram-negative rod shaped bacteria that shares a symbiotic relationship with animal host as part of normal digestive intestinal flora. Under certain define conditions these organisms or pathogenic strains of these organisms are known to induce diarrhea (Clarke, 2001; Le Bouguenec, 2005). There are six main types of pathogenic *E. coli* associated with diarrhea, namely enterotoxigenic *E. coli* (ETEC), enteroinvasive *E .coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC) and diffusively adherent *E. coli* (DAEC) (Clarke, 2001). The characteristics and mode of actions of each type of the pathological strains in diarrhea diseases are listed in Table-1.

Table-1: Charecterstice of *E. coli* starins causing diarrheal diseases

Pathogens	Epidemiology	Symptoms
Enterotoxigenic *E. coli* (ETEC)	Most common cause of traveler's diarrhea. Most of the children are affected in developing countries.	Watery diarrhea ranging from mild, self-limiting disease to severe purging.
Enteroinvasieve *E. coli* (EIEC)	Important cause of diarrhea in the areas of poor hygiene.	Bloody, mucoid diarrhea and dysentery.

Enterohaemorrhagic *E. coli* (EHEC)	Most important in human infection. Out breaks and sporadic cases all over the world.	Bloody diarrhea, fever, vomiting, haemorrhagic colitis, haemolytic uremic syndrome (HUB), acute renal failure, haemolytic anaemia
Enteropathogenic *E. coli* (EPEC)	Sporadic case and mostly occurs in baby	Self-limiting watery diarrhea with fever and vomiting.

Staphylococcus aureus

S. aureus is a gram-positive coccus present in normal intestinal and skin flora of human and homoeothermic animal. Under define conditions, the pathogenic strains produces heat stable staphylococcal enterotoxins (SETs) and toxic shock syndrome toxins (TSST-1) (de Oliveira, 2010) both of which are known to induce diarrhea. Upon ingestion of the contaminated food with the SETs, results diarrhea associated with fever, nausea and vomiting (Rosengren et al, 2010; Perez-Bosque and Moreto, 2009). In contrast, TSST-1 is characterized by sudden onset of fever, vomiting, diarrhea, erythematous rash with skin peels, hypotensive shock, impairment of renal and hepatic functions. Toxicity results by way of the production of pro-inflammatory cytokines and chemokines. Toxicity is usually aggravated by further interaction between the activated immune system and inflammatory mediators (Krakauer et al., 2001).

Campylobacter jejuni

C. jejuni is an invasive Gram-negative, spiral-shaped rod bacterium present in the GIT of mammals, birds and primates (Lengsfeld et al., 2007). The clinical signs of campylobacter infections include pyrexia, abdominal pains, watery diarrhea and dysentery (Podewils et al, 2004). The characteristic mechanisms Campylobacter infection involves invasion and translocation of the epithelium with a concomitant induction of inflammation (Hu et al., 2008).

***Shigella* species**

Four species of *Shigella* (S. *flexneri, S. dysenteriae, S. sonnei* and *S. boydii*) invades the colon with resulting inflammation and diarrhea (Podewils et al., 2004). *S. flexneri* is responsible for dysenteric symptoms and persistent illness while *S. dysenteriae* type-1 produces Shiga-toxin causes bloody diarrhea (Podewils et al, 2004), *S. sonnei* causes bacterial gastroenteritis and bacillary dysentery and *S. boydii* causes fever, chills, abdominal pain and diarrhea.

Vibrio cholerae

V. cholerae is a motile, facultative anaerobic Gram-negative rod associated with potentially fatal diarrhea (Granum, 2006) that results from the ingestion of the cholera enterotoxins (CT) (Podewils et al, 2004). Watery, colour less mucous-flecked stool and vomiting are the main clinical signs associated with cholera which in severe cases can

result in a life-threatening fluid and electrolyte imbalance (Podewils et al, 2004). Pathophysiologically, toxicity results from the CT induction of intestinal hypersecretion through the activation of the mucosal epithelium cAMP-adenylate cyclase system in the mucosal epithelium (Casburn-Jones and Farthing, 2004). Other species of *Vibrio* such as *V. parahaemolyticus* and *V. vulnificus* also caused watery diarrhea, abdominal cramps, nausea and vomiting.

***Bacillus* species**

B. cereus is a sporulating bacterium that causes diarrhea due to food poisoning resulted by enterotoxins such as haemolysin BL (HBL), non-haemolytic enterotoxin (NHE) and cytotoxin K (CytK) (Lund et al., 2000). Other species of Bacillus such as *B. subtilis, B. licheniformis, B. pumilus* and *B. megaterium* can also produce enterotoxins and emetic toxins involved in food borne illness but are usually considered relatively safe (From et al, 2007).

***Yersinia* species**

Yersinia species are Gram-negative facultative anaerobic nonsporing rods bacteria belonging to Enterobacteriaceae family. Two pathogenic species *Y. enterocolitica* and *Y. pseudotuberculosis* are responsible for yersiniosis with clinical signs such as diarrhea, vomiting, fever and abdominal pain (Linscott, 2011).

Listeria monocytogenes

L. monocytogenes is the only single species which causes life-threatening invasive diseases referred to listeriosis in human and animals (Chaturongakul et al., 2008; Todd and Notermans, 2011). Upon ingestion of the bacteria through contaminated foods, causes diarrhea by colonizing the intestinal tract (Chaturongakul et al., 2008).

***Clostridium* species**

Three species of *Clostridium* viz. *C. difficile, C. botulinum, C. perfringens* are responsible for diarrheal diseases. *C. difficile* causes a spectrum of diseases ranging from benign diarrhea to fatal colitis and most often as a consequent of antibiotics treatment. Most antibiotics predispose *C. difficile* overgrowth leading to the production and accumulation of and diarrhea are Toxins A (enterotoxin) and B (cytotoxin) in the intestine. Both toxins A and B inactivate intracellular Rhoproteins by glycosylation, leading to desorption of the cytoskeleton, production of inflammatory cytokines and damage to tight junctions. In contrast, *C. perfringens* is an important food poisoning bacterium with clinical sign as diarrhea, abdominal cramping and nausea. *C. botulinum* play a role in diarrheal diseases due to botulinum toxin resulting abdominal cramping, nausea, vomiting, diarrhea, double vision, long term nerve damage and possible even death from paralysis (Linscott, 2011).

Salmonella typhimurium

S. typhimurium is a bacterium that may be associated with mild gastroenteritis to enteric (typhoid) fever, bacteraemia and septicaemia commonly referred to as salmonellosis (Mastroeni and Maskell, 2006). The clinical signs of salmonellosis include diarrhea, fever and abdominal cramps.

Enterococcus faecalis

E. faecalis is a gram-positive bacterium that survives symbiotically in the intestinal tract. However, under stress conditions such as the disruption delicate host-commensal relationship due to overuse of antibiotic use, abdominal surgery or changes in host immunity, the enterococci becomes invaders of the intestinal wall (Butler, 2008) through the production of adhesin, aggregating and binding substances (Butler, 2008). *E. faecalis* is known to produce superoxide (O_2^-) that can results in hydroxyl radical formation which contributes to oxidative stress in the intestine and membrane lipid peroxidation (Huycke and Moore, 2002; Sun et al, 2010) resulting diarrhea.

Other bacterial species

Bacteria such as *Klebsiellae* species, *Aeromonas* species, *Enterobacter* species, *Citrobacter* species, *Proteus* species, *Pseudomonas aeruginosa* and *Plesiomonas shigelloides* were also important bacteria involved in causing diarrheal diseases.

Table-2: Clinical feuture of bacterial diarrheal disese other than *E. coli*

Pathogens	**Incubation period**	**Symptoms**
Bacillus cereus, Staphyloccus aureus	1-8 hr	Diarrhea, vomiting
Clostridium perfringens	8-24 hr	Diarrhea, abdominal cramps
Vibrio cholerae, Klebesiella pneumoniae	8-72 hr	Diarrhea, vomiting, abdominal cramps, fever
C. difficile	24-72 hr	Diarrhea, abdominal cramps
Salmonella, Campylobacter, Aeromonas, Yersania species and *V. parahaemolyticus*	12hr-11 day	Diarrhea, vomiting, abdominal cramps, fever
Shigella sp.	1-4 days	Diarrhea, abdominal cramps, fever
Giardia sp.	1-8 days	Diarrhea, fever
Rotavirus, Norvirus	24-72 hr	Diarrhea, fever

Viral agents

Several viruses belonging to Rotavirus, Norwalk viruses, Adeno viruses, Calciviruses, Coronaviruses, Astroviruses and Enteroviruses are responsible for causing diarrhea throughout the world.

Rotavirus
Rotavirus is a major cause of severe diarrhea due to the production of enterotoxin NSP4 which inducesd Na-glucose dependent malabsorption and destruction of enterocytes (cytotoxicity). The toxin also has a direct effect on the intestinal barriers by blocking TJs formation with resultant diarrhea through a 'leak flux' mechanism in which water is secreted into the lumen of the intestine (Dickman et al., 2000).

Norovirus
Norovirus is considered as major global causes of gastroenteritis resulting diarrhea (Mattison, 2010). The disease is opportunistic with clinical signs viz. nausea, vomiting, diarrhea and abdominal pains (Koopmans, 2008).

Hepatitis A virus
Hepatitis A is a small, non-enveloped spherical with cubic symmetry, thermostable and acid resistant entero virus. It multiply in the intestinal epithelium and reaches the liver. The clinical signs are dark urine, jaundice, malaise, weakness, fever, anorexia, nausea and vomiting, abdominal pains, and diarrhea (Koff, 1992).

Human immunodeficiency virus (HIV)
Chronic diarrhea is one of the complications associated with HIV infection and acquired immune deficiency syndrome (AIDS) due to multiple enteric opportunistic microbes (DuPont and Marshall, 1995). While HIV is important in secondary enteric diseases as a result of immune suppression (CD4+ T-lymphocytes destruction), the virus can result in diarrhea directly by altering the mucosa structural arrangement referred as HIVenteropathy (Epple et al., 2009). The diarrhea resulting from HIV appears to be caused by the releases of cytokines from the infected immune cells (Schmitz et al., 2002).

Protozoa and Parasitic agents
Sevral protozoans and parasites such as *Entemoeba histolytica, Giardia lamblia, Balantidium coli, Cryptosporidium* sp., *isospora belli, Fasciola hepatica, Taenia saginata, T. solium, Hymenolepis nana, Schistosoma mansoni, S. japonica, Trichuris trichiura, Ancyclostoma duodenale, Necator americanus* etc. are involved in diarrheal diseases.

Giardia intestinalis
G. intestinalis (syn. G. doudenalis, G. lamblia) is a flagellate protozoa parasite colonizes the small intestinal lumen and induces non-inflammatory and malabsorptive diarrhea (Schulzke et al., 2009). The pathophysiology of Giardiasis involves Na^+ dependent D-glucose absorption impairment, active electrogenic anion secretion activation, mucosal inflammation and leak flux (Buret, 2007; Troeger et al., 2007). Clinical signs of Giardia infection include bloating, steatorrhea and nausea.

Entamoeba histolytica

E. histolytica is a protozoa parasite which infects the large intestine with resultant intestinal dysfunction characterized by invasive illness and severe dehydration commonly referred to as amoebiasis (Ralston and Petri, 2011). The pathophysiology of amoebiasis involves villus structural destruction and increase in epithelial permeability (Lauwaet et al., 2004). The clinical signs are usually similar to *S. dysenteriae* or enteroinvasive *E. coli* with blood and pus contaminated stool. Other related infectious species include *E. dispar* and *E. moshkovskii* (Ralston and Petri, 2011).

Cryptosporidium parvum

C. parvum is an intracellular protozoa parasite that infects epithelia causing cryptosporidiosis which manifest clinically as profuse watery diarrhea containing mucus and some times blood or leukocytes (Kenny and Kelly, 2009; O'Hara and Chen, 2011).

Cyclospora cayetanensis

C. cayetanensis is a protozoan parasite which invades the epithelial cells of the small intestine (Chacin-Bonilla, 2010; Manfield and Gajadhar, 2004). The clinical signs of the infection include watery diarrhea, loss of appetite, weight loss, abdominal bloating and cramping, increased flatulence, nausea, fatigue, and low-grade fever (Linscott, 2011).

Trichinella spiralis

T. spiralis is a food-borne zoonotic parasite induced changes in intestinal function by hypersensitivity mechanism resulting in an increased intestinal chloride and fluid secretion (Cui et al., 2011). The clinical intestinal symptoms are nausea, abdominal pain, vomiting and diarrhea (Linscott, 2011).

Fungal induced diarrhea by *Candida albicans*

C. albicans is the only fungal originate organism is responsible for diarrhea and it exist as a member of normal flora in the GIT and mucocutaneous membrane. Due to overuse of antibiotic therapy that results in sterilization of the GIT flora, *C. albicans* can overgrowth to take the place of removed organisms with end result of diarrheal symptoms (Henry-Stanley et al., 2003). Other predisposing factors include altered intestinal permeability and diminished host immunity response. It has been postulated that this organism produces virulence factors which increases fungal adherence to host cells and secretion of proteolytic enzymes (Henry-Stanley et al., 2003). Clinical signs associated with enteric candidiasis are abdominal pain, cramping, rectal irritation and absence of nausea, vomiting, bloody and mucus stool, and pyrexia (Levine et al., 1995).

MATERIALS AND METHODS

The phytogeography of Similiapl Biosphere Reserve, Mayurbhanj, Odisha

The Similipal Biosphere Reserve (Figure 1), one of the mega biodiversity zones of the country, is situated in the central part of the Mayurbhanj district of Odisha (20°17′ -

22°34'N and 85°40' - 87°10'E) and covers an area of 5569 km². The reserve has been divided into three zones i.e. Core Zone, Buffer Zone and Transition Zone. The core zone (845Km²) is reserved for wildlife habitat development and no exploitation activities are entertained in this area. The buffer zone (around 2,129 Km²) is partially prohibited and is allowed for some activities like research, education and tourism. On the other hand the transition zone, which lies in the peripheral region covering 2,595 Km², is allowed for research, tribal settlement, tourism and other environment friendly activities.

Health care in the tribal villages of Mayurbhanj

In Similipal Biosphere Reserve there are 4 villages inside the core area, 65 villages in the buffer zone and 1200 villages in the peripheral zone. The human population in all being more than 4.5 lakhs, where tribals occupy about 52 % of its population and 53 communities, both aboriginal and migrated, inhabit the district glorifying the rich heritage of tribal culture. Among the tribal communities, the chief ones are Santal, Kol, Bhomij, Bhuyan, Bathuri, Kharia, Gonds, Mankdias, Pauri-Bhuyan, Saharias, Mahalis and Sounti. Some of these tribes namely Kharias, Mankdias and Saharas are still in primitive state of living. Most of the villages are situated in the mountainous terrain of the district i.e. not well communicated with the district head quarter hospital. Till now the health care system are still sparse in the core areas. The local tribal baidays and tribal old man usually are the first to prescribe medicines for various remedies when illness strikes. Regarding the common diseases the respondents informed about the frequent occurrence of skin infection with other diseases like dysentery, diarrhea and snakebite.

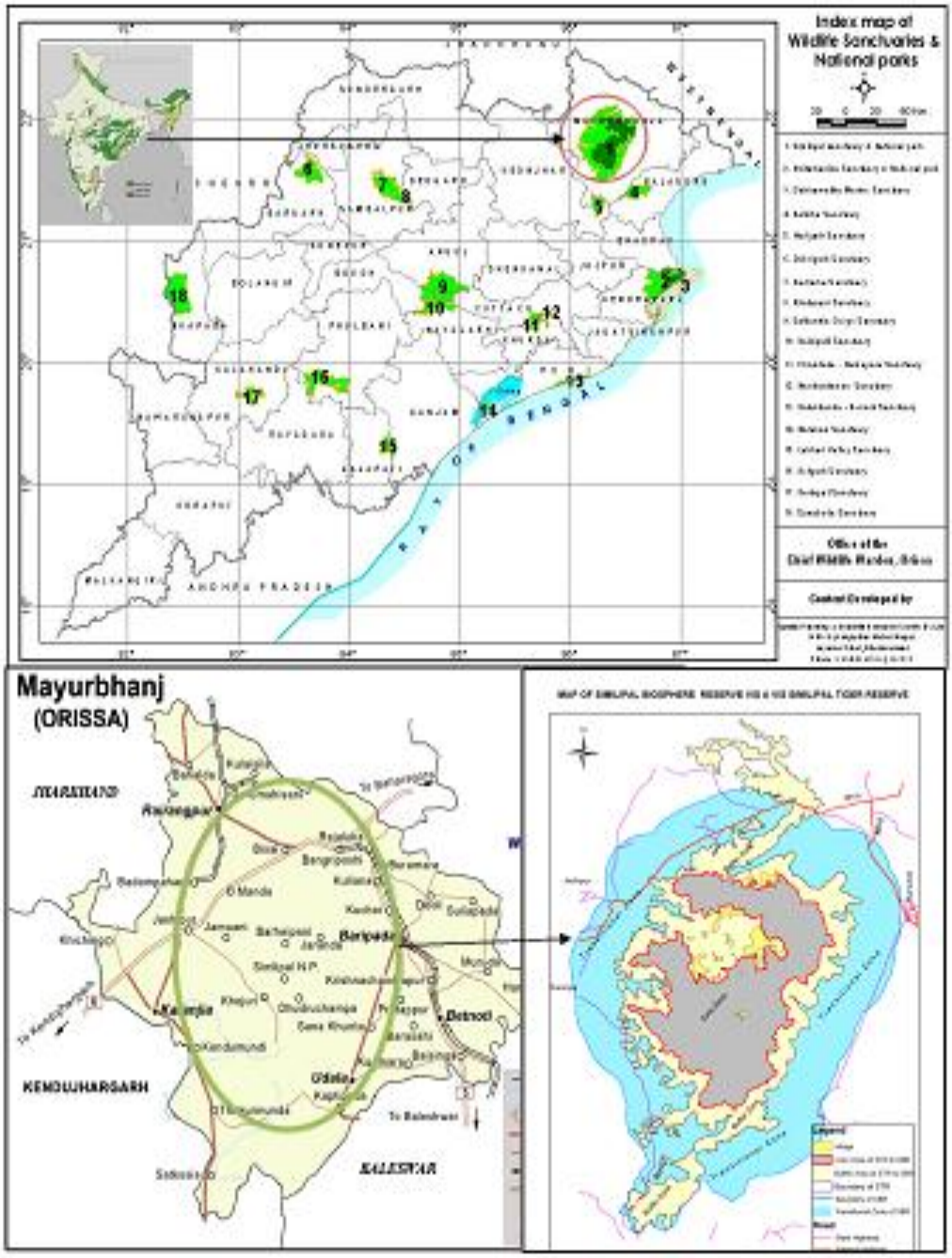


Figure-1: MAP OF Mayurbhanj District showing Similipal Biosphere Reserve

Knowledge on types of diarrheal disease among the tribes of Mayurbahanj

The most common type of diarrhea among the villagers was the green color and rice water diarrhea. All these diarrheal diseases mostly are induced by bacteria. The green color occurs because of un-processed green bile secretions from the upper small intestines which normally turn brown during transit. The black color results from blood that is acidified, as in the acidic environment of the upper small intestine

(Navaneethan and Giannella, 2011). The tribals also have also knowledge on bloody diarrhea. Most cases of bacillary dysentery, caused by *Shigella* species, lead to blood in the stool as bacteria lyse and kill the epithelial lining of the intestines (Fernandez and Sansonetti, 2003). Watery diarrhea can be caused by too many salts or fats in the colon. Watery diarrhea is caused by bacterial infection releasing toxin by *E. coli*, *Vibrio cholerae*, *V. parahaemolyticus,* or *Campylobacter jejuni*.

Ethnomedicinal documentation

In Mayurbhanj district, phytotherapy (treatment with medicines from plant and their derived products) forms an integral part of the local culture, and the information about plants and their uses are passed from generation to generation through oral folk-lore, primarily amongst the elderly; the natural retainers of traditional knowledge in their respective communities. The field study was carried out from September 2006 to November 2008, and the information on the use of medicinal plants for treatment of diarrhea was obtained through structured questionnaires, complemented by free interviews and informal conversations (Huntington, 2000). The interviews were individually carried out during the first contacts with the local population, native specialists were identified, i.e. people considered by the community as having exceptional knowledge about the use of plants.

Collection of medicinal plants

The present work is based on the explorations made in Similipal Biosphere Reserve during 2006-10. Field trips to Similipal Biosphere Reserve were undertaken for collection of medicinal plants. Identification of these medicinal plants was done at the Post Graduate Department of Botany, North Orissa University, Baripada.

Processing

Stems, leaves, bulb, barks, roots, rhizomes, seeds etc. of plants have separately been collected during field trips to different parts of Similipal Biosphere Reserve. The roots are dug out from the soil and the adhering soils were removed by shaking and washing. The leaves were plucked from the trees, washed properly and infected leaves were discarded. After collection, the healthy leaves were dried at low temperature to maintain their green color and volatile oils, if present. The materials were completely shed dried so long it does not allow for the growth of any type of fungi, molds, bacteria and other microorganisms. The dried leaves, roots and stems were powdered separately using mortar and pestle and then were passed through sieve to get the uniform powdered sample.

Preparation of plant extracts

Twenty grams of each powdered samples were dissolved in 100ml of sterile distilled water and 80% methanol separately in wide mouth bottles. The aqueous samples were

prepared by adding distilled water (steam for 30 minutes) and stored overnight. Similarly, methanol samples were incubated at room temperature for 48 hrs. The suspension was then filtered (Whatman No. 40) separately and made up to 100ml with respective solvent and utilized for studying their antimicrobial properties.

Test bacteria

Pathogenic bacteria under study were *Escherchia coli*, *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Salmonella typhi*, *Shigella dysentriae*, *Vibrio alginolyticus, Vibrio cholerae* and *Vibrio cholerae* O139.

Maintenance of bacteria

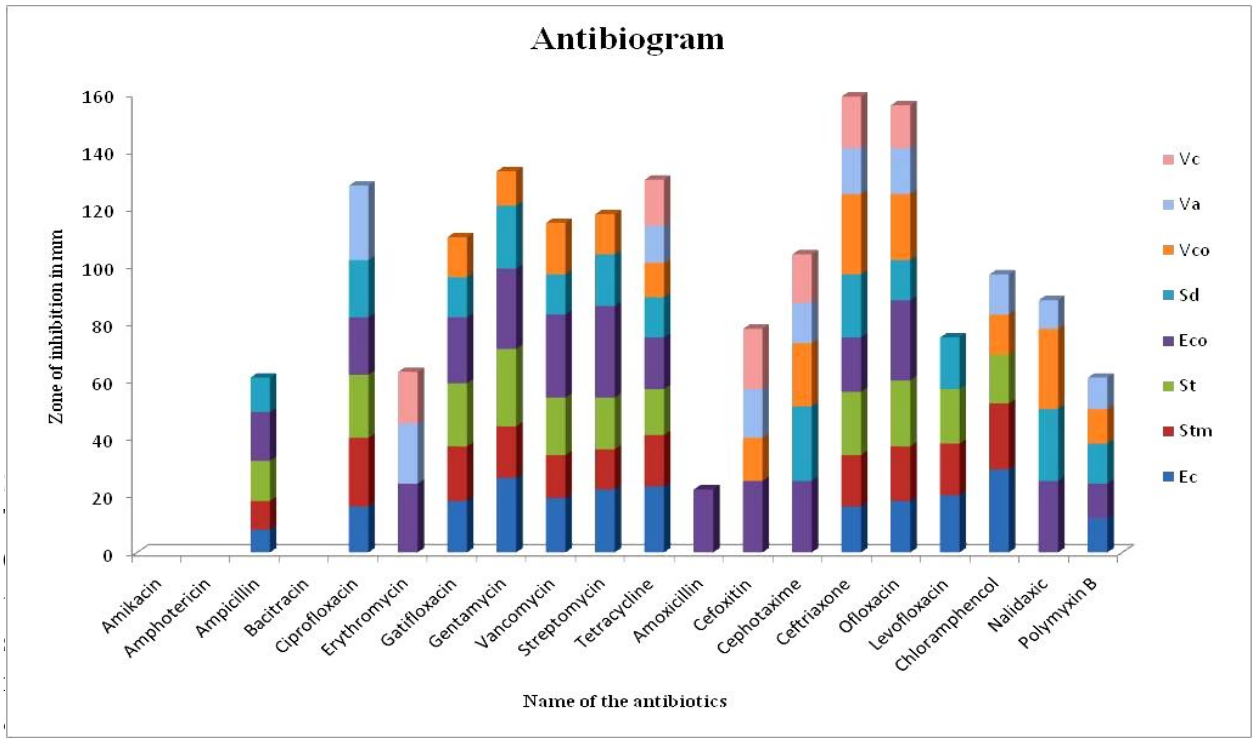
The bacterial cultures were maintained on nutrient agar (NA) slants and stored at 4 ^{o}C. Activation of the bacterial was carried out by streaking culture from the slants on to a Muller Hinton agar (MHA) plate and incubating overnight at 37 ^{o}C. Single colony was picked up from each plate and transferred to nutrient broth, incubated for 1 day at 37 ^{o}C prior to the test.

Antibiotics

The following antibiotics sensitivity test disc (Hi media Pvt. Ltd., Mumbai, India) at the given concentration were used to determine antibiotic sensitivity profile of reference bacteria: Amikacin-Ak (30µg); Amoxicillin-Aug (10µg); Amphotericin-Ap (100unit); Ampicillin-A (10µg); Bacitracin-B (10units); Cefoxitin-Ctn (10µg); Ceftriaxone-Cez (10µg); Cephotaxime-Ce (30µg); Chloroamphinecol-Ch (10µg); Ciprofloxacin-C (10µg); Erythromycin-E (15µg); Gatifloxacin-Gf (30µg); Gentamycin-G (10µg); Levofloxacin-Lvx (5µg) ; Naladixic acid-Nal (30µg); Ofloxacin-Ofl (5µg); Polymyxin-B-Pb (300 unit); Streptomycin-St (10µg); Tetracycline-Te (10µg) and Vancomycin-Vn (30 µg).

Sensitivity test

Antibiogram was done by disc diffusion method (Bauer et al., 1966) with commonly used antibiotics. Antibiotic sensitivity was tested in MHA plates. The test microbes were removed from the slant aseptically with inoculating loops and transferred to separate test tubes containing 5.0ml of sterile distilled water. Inoculums added until the turbidity equaled 0.5 McFarland (10^{8} CFU/ml). For each of the bacteria, one milliliter of the test tube suspension was added to 15-20ml of nutrient agar and transferred to the agar plate (90 mm diameter). After cooling the inoculated agars at room temperature for 25 min, antibiotic sensitivity test discs were placed on the surface of solid agar. The plates were incubated at 37°C. The plates were examined thereafter, clear zones of inhibition formed around the discs were measured and antibiotic sensitivity was assayed from the diameter of the clear inhibition zones (in mm) (Figure-2).



zone of clearance around the wells, confirmed the antibacterial activity of the extracts.

RESULTS

The antidiarrheal screening of 134 plants collected from Similipal Biosphere Reserve, Orissa, India was carried out by agar cup method. The selection of plants was based on ethnomedicinal uses reported earlier as well as freshly recorded data (Table-3). Two different solvents viz. methanolic (80%) and aqueous used for the preparation of crude extracts (Table-2). A total of 288 plants extract (Table-4) belonging to 66 families were tested for anti-diarrheal activity. Among the selected 136 plants, 100 species showed anti-bacterial activity against at least two or more test organisms. Two families with higher number of plants didn't show any activity belonged to Rubiaceae and Euphorbiaceae. Acanthaceae, Anacardiaceae, Apocynaceae, Ceasalpiniaceae, Clusiaceae, Combretaceae, Fabaceae, Moringaceae, Oleaceae, Rutaceae, Punicaceae and Verbenaceae were some of important families which showed anti-diarrheal activity against pathogens. Screening results showed antibacterial activity with 107 methanol and 74 aqueous extracts against one or more test strains.

Some of the active species have already been reported earlier to have antibacterial activity. The effects of few medicinal plants viz. *Justicia adhatoda*, *Achyranthes aspera*, *Andrographis paniculata*, *Holarrhena antidysenterica*, *Cassia fistula*, *Carica papaya*, *Punica granatum*, *Moringa oleafera*, *Vitex negundo*, *Hemidesmus Spondias pinnata* were previously described (Valsaraj et al. 1997) and was confirmed in this work. Similar results were

described by several authors by adding plants such as *Achyranthes aspera, Annona reticulate, Annona squamosa, Cassia fistula, Diospyros melanoxylon, Holarrhena pubescens, Moringa oleifera, Mesua ferrea*, *Punica grantum*, *Semecarpus anacardium, Tamarindus indica*, *Terminalia arjuna* etc. (Ahmad et al., 1998; PerumalSamy et al., 1998, 1999; Jeevan Ram et al., 2004; Prashantkumar et al., 2006; Parekh and Chanda 2008).

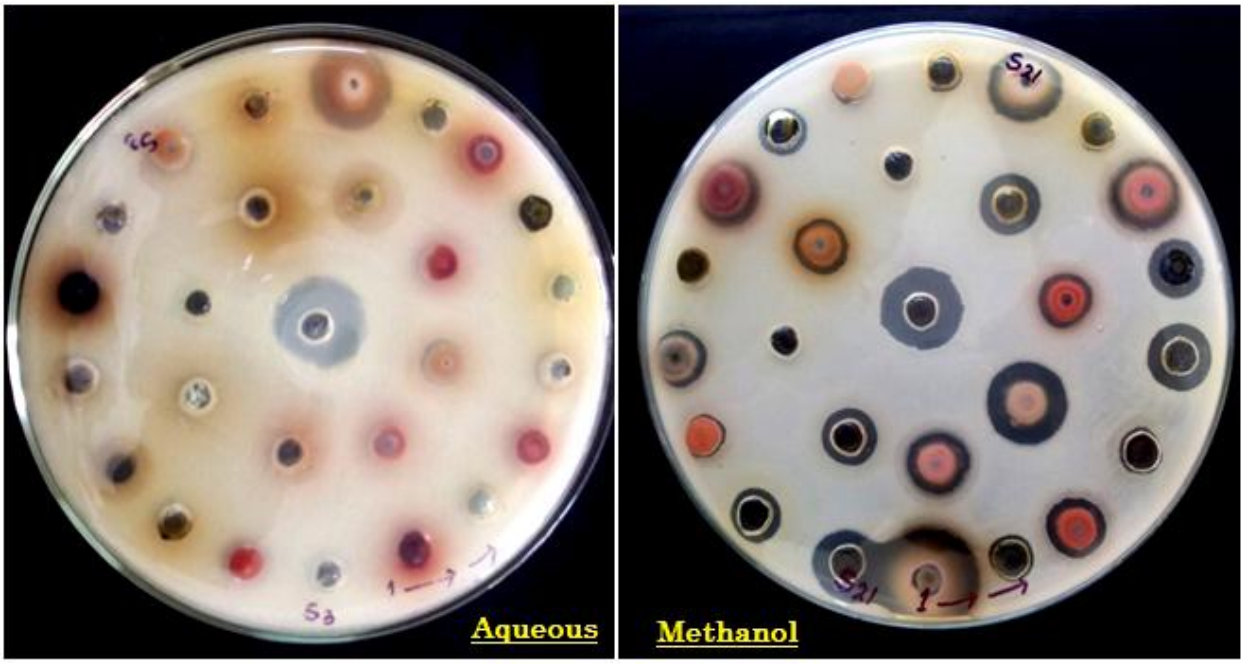


Figure 3: Agar cup method showing zone of inhibition

DISCUSSION

Diarrhea is known to be caused by several factors including the non infectious type of diarrhea such as metabolic diseases, food allergy and other organic causes. On the contrary, infectious diarrhea is a type of diarrhea which is caused by an infectious agent (bacteria, fungus, parasites and viruses) due to the invasion and colonization of the host tissue. Infectious diarrhea is characterized by an alteration in a normal bowel movement, an increase in the volume of water content, or frequency of stools, nausea, vomiting, cramps and/or abdominal discomfort (Sood and Pacheco 2002). Studies on pathogens responsible for acute diarrhea in developing countries, revealed that the contribution of Rotavirus (15-25%), entrerotoxigenic *E. coli* (10-20%), *Shigella* species (5-15%) *Salmonella* species (1-5%), *C. jejuni* (10-15%) and entropathogenic *E. coli* (1-5%) (EHNRI 2002). *E. coli* (EIEC) entroinvassive and enterohemorragic (EHEC), *Salmonella, Shigella, V. cholerae* are the major bacterial pathogens most often responsible for causing pandemic and epidemic infectious diarrheal disease in developing countries (Black et al. 1984).

Table-3: Ethnomedicinal uses of medicinal plants from district Mayurbhanj, Orissa, India

Botanical name	Family	Parts used	Cure for diseases	References
Acacia catechu (L.f.) Willd	Mimosaceae	Bk	Diarrhea	Kar et al., 2013
Acacia leucophloea (Roxb.)Willd.	Mimosaceae	Bk	Diarrhea	Panda et al., 2011a Panda et al., 2014 Kar et al., 2013
Acacia nilotica (L.) Delile	Mimosaceae	Lf	Diarrhea Dysentery	Kar et al., 2013
Achyranthes aspera L.	Amaranthaceae	Wp Rt	Dysentery	Panda et al., 2011a Rout and Panda 2010 Mohanta et al. 2006 Kar et al., 2013 Panda et al., 2014
Acorus calamus L.	Araceae	Rh	Diarrhea	Panda et al. 2011a Panda et al., 2014
Aegle marmelos L.	Rutaceae	Lf, Fr	Diarrhea	Rout and Panda 2010 Kar et al., 2013
Ageratum conyzoides L.	Asteraceae	Rt, Lf	Diarrhea, Dysentery	Kar et al., 2013
Alangium salviifolium (L.f) Wang.	Alangiaceae	Bk	Dysentery	Kar et al., 2013
Albizia lebbeck Benth.	Mimosaceae	Bk	Dysentery	Kar et al., 2013
Allium cepa L.	Liliaceae	Bl	Diarrhea, Dysentery	Rout and Panda 2010 Kar et al., 2013
Allophylus serratus (Roxb.) Kurz	Sapindaceae	Wp	Diarrhea	Kar et al., 2013
Alstonia scholaris (L.) R. Br.	Apocynaceae	Bk	Diarrhea, Dysentery	Panda et al., 2014 Kar et al., 2013

Andrographis paniculata (Burm. f.) Wall. ex Nees	Acanthaceae	Lf, St	Dysentery	Rout and Panda 2010 Kar et al., 2013
Angiopteris evecta Forst. Hoff	Angiopteridaceae	Lf	Dysentery	Panda et al. 2011a
Annona reticulata L.	Annonaceae	Lf	Diarrhea Dysentery	Padhi et al. 2011 Kar et al., 2013
Annona squamosa L.	Annonaceae	Lf	Diarrhea	Padhi et al. 2011
Anogeissus latifolia (Roxb. ex DC.) Wall. ex Bedd	Combretaceae	Lf Bk	Diarrhea	Panda et al. 2011a Mohanta et al. 2006 Panda et al., 2014 Kar et al., 2013
Anthocephalus chinensis (Lam.)A.Rich.ex.Walp.	Rubiaceae	Bk	Diarrhea	Kar et al., 2013
Ardisia solanacea Roxb.	Myrsinaceae	Rt	Diarrhea	Kar et al., 2013
Asparagus racemosus Willd.	Liliaceae	Rt	Diarrhea Dysentery	Kar et al., 2013
Azadirachta indica A.Juss.	Meliaceae	Lf	Diarrhea Dysentery	Kar et al., 2013
Barringtonia acutangula (L.) Gaertn.	Barringtoniaceae	Lf	Diarrhea Dysentery	Kar et al., 2013
Bauhinia purpuria L.	Caesalpiniaceae	Bk	Diarrhea	Kar et al., 2013
Bauhinia racemosa Lam.	Caesalpiniaceae	Bk	Dysentery	Kar et al., 2013
Bauhinia vahlii W. & A.	Caesalpiniaceae	Bk	Dysentery	Panda et al. 2011a Kar et al., 2013
Bauhinia variegata L.	Caesalpiniaceae	Bk	Dysentery	Kar et al., 2013
Boerhavia diffusa L.	Nyctaginaceae	Rt	Diarrhea Dysentery	Kar et al., 2013
Bombax ceiba L.	Bombacaceae	Gum	Diarrhea	Panda et al., 2012

Boswella serrata Roxb.	Burseraceae	Bk	Diarrhea	Kar et al., 2013
Bryophyllum calycinum Salis.	Crassulaceae	Lf	Dysentery	Panda et al., 2012
Buchanania lanzan Spreng.	Anacardiaceae	Lf	Diarrhea	Panda et al., 2012
Butea monosperma (Lam.) Taub.	Fabaceae	Rt, Sd	Dysentery	Rout and Panda 2010 Kar et al., 2013
Butea superba Roxb.	Fabaceae	Rt	Dysentery	Panda et al., 2012
Calotropis gigantea R. Br.	Asclepiadaceae	Rt	Dysentery	Panda et al., 2012 Kar et al., 2013
Calotropis procera (Ait.) R. Br.	Asclepiadaceae	Rt	Cholera	Kar et al., 2013
Canthium dicoccum (Gaertn.) Teijsm. & Binnend.	Rubiaceae	Rt Lf	Diarrhea	Kar et al., 2013
Careya arborea Roxb.	Lecythidaceae	Bk	Blood Dysentery	Mohanta et al. 2006 Rout and Panda 2010 Kar et al., 2013
Cassia fistula L.	Caesalpinaceae	Lf	Dysentery	Panda et al. 2011c
Catharanthus roseus (L.) G. Don	Apocynaceae	Lf	Dysentery	Rout and Panda 2010
Catunaregam spinosa (Thunb.) Tirven	Rutaceae	Bk	Diarrhea, Dysentery	Kar et al., 2013
Centella asiatica (L) Urban	Apiaceae	Lf	Diarrhea, Dysentery	Panda et al., 2014
Cissampelos pareira L.	Menispermaceae	Rt	Diarrhea	Rout and Panda 2010
Citrus limon (L.) Burm. f.	Rutaceae	Fr	Diarrhea	Panda et al., 2014 Kar et al., 2013
Citrus medica L.	Rutaceae	Fr	Diarrhea	Panda et al. 2011a; Rout and Panda 2010

Clausena excavata Burm. f.	Rutaceae	Rt, Lf	Diarrhea, Dysentery	Panda et al. 2011a Mohanta et al. 2006 Panda et al., 2014 Kar et al., 2013
Cleome viscosa L.	Capparaceae	Wp	Diarrhea	Kar et al., 2013
Commelina benghalensis L.	Commelinaceae	Wp	Dysentery	Kar et al., 2013
Coccinia grandis (L.) Voigt	Cucurbitaceae	Lf	Diarrhea, Dysentery	Panda et al., 2012
Crotalaria spectabilis Roth.	Fabaceae	Rt	Dysentery	Rout and Thatoi, 2009 Panda et al., 2014
Croton roxburghii Balak.	Euphorbiaceae	Lf	Dysentery	Panda et al. 2010 Panda et al., 2014
Curculigo orchioides Gaertn.	Hypoxidaceae	RT	Dysentery	Panda et al., 2012
Curcuma angustifolia Roxb.	Zingiberaceae	Rh	Dysentery	Panda et al. 2011a Mohanta et al. 2006 Panda et al., 2014 Kar et al., 2013
Curcuma aromatica Salisb.	Zingiberaceae	Rh	Dysentery	Panda et al., 2014
Cynodon dactylon (L.) Pers.	Poaceae	Wp	Diarrhea	Panda et al. 2011a Kar et al., 2013 Panda et al., 2014
Cyperus rotundus L.	Cyperaceae	Rh	Diarrhea	Kar et al., 2013
Desmodium gangeticum (L.) DC.	Fabaceae	Rt	Dysentery	Panda et al., 2014
Diospyros malabarica (Desr.) Kostel	Ebenaceae	Bk	Diarrhea	Panda et al. 2011a Kar et al., 2013
Diospyros melanoxylon	Ebenaceae	Lf, Bk	Diarrhea,	Panda et al. 2009

Roxb.			Dysentery	Kar et al., 2013
Elephantopus scaber L.	Asteraceae	Rt, Lf	Diarrhea	Kar et al., 2013
Eleutherine bulbosa (Miller) Urban	Iridaceae	Bl	Dysentery	Panda et al., 2012
Emilia sonchifolia (L.) DC	Asteraceae	Wp	Diarrhea	Kar et al., 2013
Erycibe paniculata Roxb.	Convolvulaceae	Bk	Diarrhea, Dysentery	Panda et al., 2014 Kar et al., 2013
Eryngium foetidum L.	Apiaceae	Lf	Diarrhea	Panda et al., 2014
Ficus benghalensis L.	Moraceae	Bk	Dysentery	Kar et al., 2013
Ficus racemosa L.	Moraceae	Bk	Dysentery Diarrhea	Panda et al., 2012 Kar et al., 2013
Flemingia nana Roxb.	Fabaceae	Rt	Diarrhea, Dysentery	Panda et al., 2012 Panda et al., 2014
Gardenia gummifera L. f.	Rubiaceae	Rs	Diarrhea, Dysentery	Kar et al., 2013
Gardenia resinifera Roth.	Rubiaceae	Rs	Diarrhea, Dysentery	Kar et al., 2013
Glycyrrhiza glabra L.	Fabaceae	Bk	Diarrhea	Rout and Panda 2010
Grewia abutilifolia Vent.ex A.L.Juss	Tiliaceae	Lf Bk	Dysentery	Panda et al., 2012 Kar et al., 2013
Grewia helicterifolia Wall.ex.G.Don	Tiliaceae	Fr	Diarrhea, Dysentery	Kar et al., 2013
Haldinia cordifolia (Roxb.) Ridsd.	Rubiaceae	Rt	Diarrhea, Dysentery	Kar et al., 2013
Helicteres isora L.	Sterculiaceae	Rt	Diarrhea, Dysentery	Panda et al., 2012 Kar et al., 2013
Hibiscus rosasinensis L.	Malvaceae	Tw	Dysentery	Panda et al. 2011a Mohanta et al. 2006 Kar et al., 2013

				Panda et al., 2014
Holarrhena pubescens (Buch.-Ham.) Wall ex. G.	Apocyanaceae	Lf	Dysentery	Panda et al. 2011a Mohanta et al. 2006 Kar et al., 2013 Panda et al., 2014
Hyptis suaveolens (L.) Poit.	Lamiaceae	Wp	Dysentery	Panda et al., 2014
Indigofera cassioides Rottl. ex DC.	Fabaceae	Rt, Fl	Dysentery	Panda et al. 2011a Mohanta et al. 2006 Kar et al., 2013 Panda et al., 2014
Ixora pavetta Andr.	Rubiaceae	Bk	Dysentery	Panda et al., 2014 Kar et al., 2013
Justicia adhatoda L.	Acanthaceae	Lf	Diarrhea	Rout and Panda 2010 Kar et al., 2013
Kalanchoe pinnata (Lam.) Pers.	Crassulaceae	Lf	Dysentery	Panda et al. 2011a Mohanta et al. 2006 Panda et al., 2014 Kar et al., 2013
Lannea coromandelica Houtt.	Anacardiaceae	Lf	Diarrhea, Dysentery	Panda et al., 2012 Kar et al., 2013
Lawsonia inermis L.	Lythraceae	Rt, Lf	Diarrhea, Dysentery	Kar et al., 2013
Leea indica (Burm.f.) Merr.	Rutaceae	Fr	Diarrhea, Dysentery	Kar et al., 2013
Limonia acidissima L.	Rutaceae	Fr	Diarrhea, Dysentery	Kar et al., 2013
Litsea glutinosa (Lour.) Robin	Louraceae	Bk	Diarrhea, Dysentery	Kar et al., 2013
Lygodium flexuosum L.	Lygodiaceae	Wp	Blood	Panda et al. 2011a

			Dysentery	
Lygodium microphyllum (cav.)R.Br.	Lygodiaceae	Lf	Dysentery	Panda et al. 2011a
Mangifera indica L.	Anacardiaceae	Bk	Blood Dysentery	Panda et al. 2011a Rout and Panda 2010 Kar et al., 2013 Panda et al., 2014
Melastoma malabathricum L.	Melastomataceae	Lf	Diarrhea Dysentery	Kar et al., 2013
Mesua ferrea L.	Clusiaceae	Lf	Diarrhea Dysentery	Panda et al., 2012
Mimusops elengi L	Sapotaceae	Bk	Diarrhea, Dysentery	Kar et al., 2013
Momordica charantia L.	Dipterocarpaceae	Bk	Diarrhea, Dysentery	Mohanta et al. 2006
Morinda citrifolia L.	Rubiaceae	Rt	Dysentery	Panda et al. 2011a Panda et al., 2014 Kar et al., 2013
Moringa oleifera Lam.	Moringaceae	Lf	Diarrhea	Mohanta et al. 2006 Kar et al., 2013
Murraya koenigii (L.) Spreng	Rutaceae	Lf	Diarrhea, Dysentery	Kar et al., 2013
Musa paradisica L.	Musaceae	Fr	Dysentery	Mohanta et al. 2006 Panda, 2010
Nicotiana tabacum L.	Solanaceae	Wp	Diarrhea	Rout and Panda 2010 Kar et al., 2013
Nyctanthes arbortristis L.	Oleaceae	Bk	Dysentery	Panda et al. 2011b Rout and Panda 2010 Panda et al., 2014

Ocimum canum Sims	Lamiaceae	Lf	Dysentery	Kar et al., 2013
Ocimum sactum L.	Lamiaceae	Lf	Diarrhea	Kar et al., 2013
Oroxylum indicum (L.) Vent.	Bignoniaceae	Lf, Bk	Dysentery	Kar et al., 2013
Oxalis corniculata L.	Oxalidaceae	Wp	Diarrhea, Dysentery	Panda et al., 2012
Paederia foetida L.	Rubiaceae	Lf	Diarrhea	Panda et al. 2011b Panda et al., 2014
Phyllanthus emblica L.	Euphorbiaceae	Lf	Diarrhea, Dysentery	Kar et al., 2013
Phyllanthus fraternus Webster	Euphorbiaceae	Wp	Diarrhea	Panda et al., 2012 Kar et al., 2013
Piper longum L.	Piperaceae	Wp	Cholera	Kar et al., 2013
Piper nigrum L.	Piperaceae	Sd, Fr	Diarrhea	Rout and Panda 2010
Plumbago zeylanica L.	Plumbaginaceae	Rt	Diarrhea	Rout and Panda 2010 Kar et al., 2013
Pogostemon benghalensis (Burm.f.) Kuntze	Lamiaceae	Lf	Dysentery	Kar et al., 2013
Psidium guava L.	Myrtaceae	Lf, Fr	Diarrhea	Rout and Panda 2010 Kar et al., 2013
Pterocarpus marsupium Roxb.	Dipterocarpaceae	Bk	Blood Dysentery	Panda et al. 2011 Mohanta et al. 2006 Panda et al., 2014 Kar et al., 2013 Rout and Panda 2010
Pterocarpus santalinus L. f.	Fabaceae	Fr	Dysentery	Kar et al., 2013
Pterospermum acerifolium (L.) Willd.	Sterculiaceae	Bk	Diarrhea Dysentery	Panda and Dutta, 2011 Panda et al., 2014

Punica granatum L.	Punicaceae	Lf	Dysentery	Pandey and Rout 2002 Kar et al., 2013
Quisqualis indica L.	Combretaceae	Sd	Diarrhea	Kar et al., 2013
Randia uliginosa (Retz.) DC	Rubiaceae	Fr	Dysentery	Panda et al., 2012
Rauvolfia serpentina (L.) Benth.ex.Kurz.	Apocynaceae	Rt	Diarrhea	Kar et al., 2013
Rubia cordifolia L.	Rubiaceae	Rt	Diarrhea Dysentery	Kar et al., 2013
Saraca asoca (Roxb.) de Wilde	Caesalpiniaceae	Bk, Lf	Dysentery	Kar et al., 2013
Schleichera oleosa (Lour.)	Sapendaceae	Wp	Diarrhea	Panda et al. 2011a
Securinega virosa (Roxb. ex Willd). Baill	Euphorbiaceae	Bk	Diarrhea	Kar et al., 2013
Semecarpus anacardium L.f.	Anacardiaceae	Fr	Diarrhea	Panda et al., 2012
Sesamum orientali L.	Pedaliaceae	Lf	Cholera, Dysentery	Kar et al., 2013
Shorea robusta Gaertn. F.	Dipterocarpaceae	Bk	Dysentery	Mohanta et al. 2006 Kar et al., 2013
Sida acuta Burm.f.	Malvaceae	Lf	Dysentery	Kar et al., 2013
Smilax zeylanica L.	Smilacaceae	Rt	Diarrhea	Panda et al., 2012
Soymida febrifuga (Roxb.) A. Juss.	Melastomataceae	Bk	Dysentery	Rout and Panda 2010
Spilanthes calva DC.	Asteraceae	Fl	Dysentery	Kar et al., 2013
Spondias pinnata (L.f.) Kurz	Anacardiaceae	Lf, Fr, Bk	Dysentery	Panda et al. 2011a Mohanta et al. 2006 Kar et al., 2013

				Panda et al., 2014
Streblus aspera Lour.	Moraceae	Bk	Dysentery	Kar et al., 2013
Strychnos nux-vomica L.	Strychnaceae	Rt	Cholera	Kar et al., 2013
Syzygium cumuni L. Skeels	Myrtaceae	Bk	Dysentery	Mohanta et al. 2006
			Diarrhea	Kar et al., 2013
Tamarindus indica L.	Caesalpiniaceae	Lf, Sd	Diarrhea	Rout and Panda 2010
Tamilnadia uliginosa (Retz.) Tirveng.	Rubiaceae	Rt	Diarrhea Dysentery	Kar et al., 2013
Terminalia alata Heyne ex. Roth	Combretaceae	Bk	Diarrhea	Mohanta et al. 2006
				Kar et al., 2013
Terminalia arjuna (Roxb. ex DC.) W. & A.	Combretaceae	Bk	Chronic Dysentery	Pandey and Rout 2002
				Kar et al., 2013
				Rout and Panda 2010
Terminalia bellirica (Gartn.) Roxb.	Combretaceae	Bk	Dysentery	Kar et al., 2013
Terminalia chebula Retz.	Combretaceae	Bk	Diarrhea	Rout and Panda 2010
Thalictrum foliolosum DC.	Ranunculaceae	Rh	Dysentery	Kar et al., 2013
Tinospora cordifolia (Willd.) Hook.f.& Thoms.	Menispermaceae	Rt, St	Diarrhea Dysentery	Kar et al., 2013
Tragia involucrate L.	Euphorbiaceae	Wp	Diarrhea	Rout et al. 2009
Trapa natans L.	Trapaceae	Fr	Diarrhea	Panda et al., 2014
Trema orientalis (L.) Bl.	Ulmaceae	Rt	Diarrhea	Kar et al., 2013
Trewia nudiflora L.	Euphorbiaceae	Lf	Dysentery	Kar et al., 2013
Tridax procumbens L.	Asteraceae	Rt	Diarrhea	Kar et al., 2013
Vitex negundo L.	Verbenaceae	Lf	Diarrhea	Mohanta et al. 2006
				Kar et al., 2013
Woodfordia fruticosa (L.) Kurz	Lythraceae	Rt	Dysentery	Panda et al. 2011a
				Rout and Thatoi 2009

				Kar et al., 2013 Panda et al., 2014
Wrightia tinctoria (Roxb.) R. Br.	Apocynaceae	Bk	Diarrhea	Kar et al., 2013
Zingiber officinale Rosc.	Zingiberaceae	Rh	Diarrhea	Kar et al., 2013
Ziziphus mauritiana Lam.	Rhamnaceae	Bk	Dysentery	Kar et al., 2013

Bl-bulb; Bk-bark; Fl-flower; Fr-fruit; Lf-leaf; Rt-root; Rh-rhizome; Rs-Resin; Sd-seed; Tw-twig; Wp-whole plant

A total of 288 plants extracts (Table-2) belonging to 66 families were tested for anti-diarrheal activity. Among the selected 136 plants, 100 species showed anti-bacterial activity against at least two or more test organisms. Some of important families with anti-diarrheal activity against pathogens are Acanthaceae, Anacardiaceae, Apocynaceae, Ceasalpiniaceae, Clusiaceae, Combretaceae, Fabaceae, Moringaceae, Oleaceae, Rutaceae, Punicaceae and Verbenaceae. Screening results showed antibacterial activity with 107 methanol and 74 aqueous extracts against one or more test strains. Solvent or the extraction agent used in the preparation of phytopharmaceuticals must be suitable for dissolving the important therapeutic drug constituents. In addition, solvents used should be easy to remove, inert, nontoxic, and not easily flammable. The aqueous extracts have commonly been used in preliminary studies. Methanol efficiently penetrates cell membranes, permitting the extraction of high amounts of endocellular components in contrast to the solvents with lower polarity. Such solvents are limited for extracting mostly extracellular material. Hence, methanol chiefly dissolves polar constituents together with medium and low polarity compounds extracted by co-solubilazation. So, the aqueous and methanolic (80%) extracts of different plant belonging to a wide range of families are selected for screening based on random sampling and observed ethnomedicinal uses.

Table-4: Preliminary screening of plants against diarrhea causing bacteria

Plant description	PU	E	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8
Acanthaceae										
Adhatoda vasica Nees	Lf	A	12	-	11	-	-	12	12	12
		M	11	12	10	12	-	11	10	10
Andrographis paniculata	Lf	A	11	-	-	11	12	12	12	10
(Burm. f.) Wall. ex Nees		M	14	-	-	-	-	-	-	13
	St	A	12	12	-	-	-	-	-	12
		M	13	12	10	12	12	12	10	-
Alangiaceae										
Alangium salviifolium (L.f)	Lf	A	-	-	-	-	-	-	11	12
Wang.		M	12	-	-	-	-	-	10	13
Amaranthaceae										
Achyranthes aspera L.	Wp	A	-	-	9	-	-	-	-	11
		M	-	10	8	-	-	-	-	12
Anacardiaceae										
Buchanania lanzan	Lf	A	-	-	-	-	-	-	-	-
Spreng.		M	10	-	-	12	12	14	-	13
Lannea coromandelica	Lf	A	12	-	-	10	-	-	10	9

(Houtt.) Merr.		M	14	12	-	14	-	12	10	14
Mangifera indica L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	12	-	-	-	-	10
Semecarpus anacardium	Fr	A	-	13	-	-	-	12	-	12
L.f.		M	12	11	-	-	14	12	-	13
Spondias pinnata	Lf	A	-	-	-	-	-	-	-	-
(L.f.) Kurz		M	12	-	13	-	-	-	-	12
	Fr	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Angiopteridaceae										
Angiopteris evecta Forst.	Lf	A	-	-	11	-	-	-	-	-
Hoff		M	-	-	14	-	-	-	-	-
Annonaceae										
Annona reticulata L.	Lf	A	-	-	12	-	-	-	-	-
		M	12	-	12	-	-	-	12	-
Annona squamosa L.	Lf	A	12	-	-	-	-	-	-	-
		M	11	-	-	-	-	11	12	-
Apiaceae										
Centella asiatica (L) Urban	Wp	A	11	-	10	-	-	-	11	-
		M	12	-	14	12	-	-	12	12
Eryngium foetidum L.	Lf	A	10	-	-	11	12	-	-	13
		M	11	-	-	11	12	-	-	13
Apocynaceae										
Alstonia scholaris (L.) R.	Lf	A	-	-	-	-	-	-	11	-
Br.		M	12	-	10	-	-	-	11	14
Catharanthus roseus	Lf	A	-	-	-	-	-	-	-	-
(L.) G. Don		M	-	-	-	-	-	-	-	-
Holarrhena pubescens	Lf	A	-	11	-	11	12	11	-	14
(Buch.-Ham.) Wall ex. G.		M	-	10	12	12	-	-	-	14
Rauvolfia serpentina (L.)	Lf	A	-	-	-	-	-	-	-	-
Benth. ex. Kurz.		M	-	-	-	-	-	-	-	-
Wrightia tinctoria (Roxb.)	ND									
R. Br.										
Araceae										
Acorus calamus L.	Rh	A	-	-	09	-	-	-	12	-
		M	-	-	12	12	-	-	14	11
Asclepiadaceae										
Calotropis gigantea L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Calotropis procera (Ait.)	Lt	A	-	-	12	-	-	-	11	-

R. Br.		M	-	-	12	-	-	-	10	-
Asteraceae										
Ageratum conyzoides L.	Wp	A	12	12	-	-	-	-	-	11
		M	-	12	-	-	-	-	-	-
Elephantopus scaber L.	Lf	A	-	-	-	-	8	-	-	11
		M	-	-	10	-	10	-	-	14
Emilia sonchifolia (L.) DC	ND									
Spilanthes calva DC.	ND									
Tridax procumbens L.	Lf	A	10	-	13	-	-	-	-	-
		M	12	-	12	-	-	-	-	-
Barringtoniaceae										
Barringtonia acutangula (L.) Gaertn.	ND									
Bombacaceae										
Bombax ceiba L.	Gum	A	-	-	-	-	-	-	-	-
		M	10	-	-	-	-	-	12	-
Bignoniaceae										
Oroxylum indicum (L.) Vent.	Bk	A	10	-	-	-	-	-	-	-
		M	10	12	-	-	-	-	-	13
Burseraceae										
Boswella serrata Roxb.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Caesalpiniaceae										
Bauhinia purpuria L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	11	-	-	-	-	-
Bauhinia racemosa Lam.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Bauhinia variegata L.	Lf	A	-	10	-	-	-	-	-	-
		M	-	12	12	-	-	-	-	-
Bauhinia vahlii W. & A.	Lf	A	-	-	-	-	-	-	-	-
		M	-	12	10	-	-	-	-	-
Cassia fistula L.	Lf	A	9	12	12	11	12	12	11	9
		M	13	12	13	12	12	12	10	12
Saraca asoca (Roxb.) de Wilde	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	13	-	-	-	-	-
Tamarindus indica L.	Lf	A	-	-	12	-	-	-	-	10

		M	12	-	14	-	-	-	-	12
Capparaceae										
Cleome viscosa L.	Lf	A	10	-	-	-	-	-	-	10
		M	-	-	-	-	-	-	-	11
Clusiaceae										
Mesua ferrea L.	Lf	A	12	10	-	-	12	-	12	12
		M	10	12	-	-	14	-	12	10
Combretaceae										
Anogeissus latifolia (Roxb.	Lf	A	10	-	-	-	12	12	12	12
ex DC.) Wall. ex Bedd		M	10	14	11	12	15	14	12	12
Quisqualis indica L.	Lf	A	10	12	-	-	12	-	12	12
		M	12	15	14	-	12	12	15	12
Terminalia alata	Lf	A	-	-	-	-	-	-	-	-
Heyne ex. Roth		M	-	-	12	-	-	-	-	-
Terminalia arjuna	Bk	A	10	12	12	12	12	-	14	12
(Roxb. ex DC.) W. & A.		M	12	15	14	12	12	12	15	12
Terminalia bellirica (Gartn.)	Bk	A	11	13	-	-	-	-	-	-
Roxb.		M	14	12	-	-	10	11	12	-
Terminalia chebula Retz.	Bk	A	-	-	-	14	12	-	-	-
		M	-	-	-	12	11	-	-	-
Commelinaceae										
Commelina benghalensis L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Erycibe paniculata Roxb.	Lf	A	10	-	10	-	-	-	-	12
		M	10	12	14	-	14	-	-	17
Crassulaceae										
Bryophyllum calycinum	Lf	A	-	-	-	-	-	-	-	-
Salis.		M	-	-	-	-	-	-	-	-
Kalanchoe pinnata	Lf	A	-	-	-	-	-	-	-	-
(Lam.) Pers.		M	-	-	-	-	-	-	-	-
Cucurbitaceae										
Coccinia grandis	Lf	A	12	11	-	-	-	-	-	11
(L.) Voigt		M	-	14	-	-	-	-	-	12
Momordica charantia L.	Lf	A	-	12	-	-	-	-	12	-
		M	-	14	-	-	-	-	-	-
Cyperaceae										
Cyperus rotundus L.	Lf	A	-	10	-	-	-	-	-	-
		M	-	12	-	-	-	-	-	10
Dipterocarpaceae										

Shorea robusta Gaertn. f.	Lf	A	-	-	11	-	12	-	-	-
		M	11	-	13	-	9	-	-	12
Ebenaceae										
Diospyros malabarica	Bk	A	-	-	-	-	-	-	-	-
(Desr.) Kostel		M	10	-	-	-	-	-	-	12
Diospyros melanoxylon	Lf	A	-	-	-	-	-	-	-	11
Roxb.		M	14	-	-	-	-	-	-	12
	Bk	A	-	-	11	11	10	-	-	12
		M	12	15	10	10	10	-	-	16
Euphorbiaceae										
Croton roxburghii	Lf	A	17	-	13	-	-	-	12	-
Balak.		M	14	10	10	12	14	-	15	15
	Bk	A	-	-	-	10	-	-	-	14
		M	-	-	-	-	-	-	-	15
Phyllanthus emblica L.	Fr	A	12	10	-	-	-	-	-	12
		M	12	-	-	12	10	-	-	15
Phyllanthus fraternus	Lf	A	-	-	11	-	-	-	-	-
Webster		M	-	-	-	-	-	-	-	-
Securinega virosa (Roxb. ex Willd). Baill	ND									
Tragia involucrate L.	Wp	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Trewia nudiflora L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Fabaceae										
Butea monosperma	Lf	A	-	-	-	-	-	-	-	-
(Lam.) Taub.		M	12	-	12	-	-	-	-	12
	Fl	A	10	-	11	-	-	-	-	12
		M	12	-	12	10	-	-	-	14
Butea superba Roxb.	Lf	A	10	10	-	-	-	-	-	10
		M	10	12	-	-	-	-	-	-
Crotalaria spectabilis Roth	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Desmodium gangeticum (L.)	Lf	A	8	-	-	-	-	-	-	10
DC.		M	10	10	-	-	-	-	-	12
Flemingia nana Roxb.	Rt	A	-	-	10	-	-	-	-	-
		M	10	-	12	-	-	-	-	10
Glycyrrhiza glabra (L.)	Bk	A	-	-	10	-	-	-	-	-
		M	-	-	18	-	-	-	-	-
Indigofera cassioides	Lf	A	-	-	-	-	-	-	-	-

Rottl. ex DC.		M	-	10	10	8	-	-	-	10
Pterocarpus marsupium	Bk	A	-	10	-	-	-	-	-	10
Roxb.		M	10	12	-	-	9	-	-	12
Pterocarpus santalinus L. f.	Bk	A	-	10	-	-	-	-	-	-
		M	10	12	-	-	9	-	-	-
Hypoxidaceae										
Curculigo orchioides	Rt	A	-	-	-	-	-	-	-	-
Gaertn.		M	-	-	-	-	-	-	-	-
Iridaceae										
Eleutherine bulbosa	Bl	A	-	14	-	-	-	-	-	17
(Miller) Urban		M	12	15	-	-	-	-	11	15
Lamiaceae										
Hyptis suaveolens (L.) Poit.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	10	-	-	-	-	-
Ocimum canum Sims	Lf	A	-	-	-	-	-	-	-	10
		M	12	-	-	-	-	-	-	12
Ocimum sactum L.	Lf	A	-	10	-	-	-	-	-	10
		M	10	10	-	-	-	-	-	10
Pogostemon benghalensis (Burm.f.) Kuntze	ND									
Lauraceae										
Litsea glutinosa (Lour.)	Lf	A	-	-	10	-	-	-	-	-
Robin		M	10	11	12	-	-	-	-	-
Lecythidaceae										
Careya arborea Roxb.	Lf	A	-	-	-	10	-	-	-	-
		M	12	-	12	12	-	15	-	14
Liliaceae										
Allium cepa L.	Bl	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Asparagus racemosus	Rt	A	-	-	-	-	-	-	-	-
Willd.		M	-	-	-	-	-	-	-	-
Lygodiaceae										
Lygodium flexuosum L.	Wp	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Lygodium microphyllum	Lf	A	-	-	-	-	-	-	-	-

(cav.) R. Br.		M	-	-	-	-	-	-	-	-
Lythraceae										
Lawsonia inermis L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	12	-	-	-	-	-
Woodfordia fruticosa	Lf	A	-	-	-	-	-	-	-	12
(L.) Kurz		M	-	-	11	-	-	-	-	14
Malvaceae										
Hibiscus rosasinensis L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Sida acuta Burm.f.	Lf	A	-	-	10	-	-	-	-	-
		M	-	-	13	-	-	-	-	-
Melastomataceae										
Melastoma malabathricum L	Bk	A	-	-	10	-	-	-	-	-
		M	-	-	20	-	-	12	-	16
Soymida febrifuga	Lf	A	-	-	-	-	-	-	-	-
(Roxb.) A. Juss.		M	-	-	-	-	-	-	-	-
Meliaceae										
Azadirachta indica A. Juss.	Bk	A	11	-	13	-	-	-	-	12
		M	10	-	-	-	-	-	-	12
Menispermaceae										
Cissampelos pareira L.	Rt	A	-	-	-	-	-	-	-	12
		M	12	-	-	14	-	10	10	14
Tinospora cordifolia (Willd.)	Wp	A	-	-	-	-	-	-	-	12
Hook.f.& Thoms.		M	10	-	13	11	-	10	10	14
Mimosaceae										
Acacia catechu (L.f.) Willd	ND									
Acacia leucophloea	Lf	A	-	10	-	-	-	-	-	9
(Roxb.)Willd.		M	10	12	10	-	-	-	12	14
Acacia nilotica (L.) Delile	ND									
Albizia lebbeck Benth.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Moraceae										
Ficus benghalensis L.	Lf	A	-	-	-	-	-	-	-	-

		M	-	-	-	-	-	-	-	-
Ficus racemosa L.	Lf	A	12	-	-	-	-	-	-	-
		M	-	-	14	-	-	-	-	12
Streblus aspera Lour.	ND									
Moringaceae										
Moringa oleifera Lam.	Lf	A	12	10	8	-	-	-	-	-
		M	14	11	12	-	-	-	10	12
Musaceae										
Musa paradisiaca L.	Fl	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Myrsinaceae										
Ardisia solanacea Roxb.	Lf	A	-	-	-	-	-	-	-	-
		M	10	10	-	-	-	-	-	-
Myrtaceae										
Psidium guajava L.	Lf	A	-	-	-	-	10	-	-	12
		M	-	-	-	-	13	-	-	14
Syzygium cumunis L.	Bk	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Nyctaginaceae										
Boerhavia diffusa L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	9	-	-	-	-	-
Oleaceae										
Nyctanthes arbortristis L.	Lf	A	12	-	10	-	-	10	-	12
		M	20	-	13	10	-	12	-	10
	Bk	A	12	10	10	12	-	12	-	14
		M	23	10	18	12		12		11
Oxalidaceae										
Oxalis corniculata L.	Wp	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Pedaliaceae										
Sesamum orientali L.	Wp	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Piperaceae										
Piper longum L.	Sd	A	10	10	-	12	-	10	10	10
		M	13	13	10	12	-	12	13	14
Piper nigrum L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Plumbaginaceae										
Plumbago zeylanica L.	Lf	A	-	-	-	-	-	-	-	-

		M	-	-	-	-	-	-	-	-
Cynodon dactylon	Wp	A	-	-	-	-	-	-	-	-
(L.) Pers.		M	-	-	-	-	-	-	-	-
Punicaceae										
Punica granatum L.	Lf	A	10	10	14	-	10	-	-	12
		M	-	-	12	-	12	-	-	10
Ranunculaceae										
Thalictrum foliolosum DC.	ND									
Ziziphus mauritiana Lam.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	9	-	-	-	-	10
Rubiaceae										
Anthocephalus chinensis	Lf	A	12	-	-	-	-	-	10	-
(Lam.)A. Rich. ex. Walp.		M	12	-	14	-	12	-	-	-
Canthium dicoccum	Lf	A	-	-	-	-	-	-	-	-
(Gaertn.) Teijsm. & Binnend.		M	-	-	-	-	-	-	-	-
Gardenia gummifera L. f.	Lf	A	-	-	-	-	-	-	-	-
		M	-	10	10	-	-	-	-	-
Gardenia resinifera Roth.	ND									
Haldinia cordifolia (Roxb.)	Lf	A	-	-	-	-	-	-	-	-
Ridsd		M	10	10	12	-	-	-	-	12
Ixora pavetta Andr.	Lf	A	10	-	-	-	-	-	-	-
		M	12	-	-	-	-	-	-	-
Morinda citrifolia L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Paederia foetida L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Randia uliginosa	Lf	A	-	-	-	-	-	-	-	-
(Retz.) DC		M	-	-	-	-	-	-	-	-
Rubia cordifolia L.	Lf	A	-	-	-	-	-	-	-	10
		M	10	-	-	-	-	-	-	12
Tamilnadia uliginosa (Retz.) Tirveng.	ND									
Rutaceae										
Aegle marmelos L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-

	Fr	A	-	-	-	-	-	-	-	-
		M	-	-	12	-	10	-	-	12
Catunaregam spinosa	Lf	A	-	-	-	-	-	-	-	-
(Thunb.) Tirven		M	12	-	12	-	-	-	-	-
Citrus limon L.	Fr	A	-	-	10	-	-	-	-	-
		M	10	-	12	-	-	-	-	11
Citrus medica L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	9	-	-	-	-	-
Clausena excavata Burm.	Lf	A	-	-	-	-	-	-	-	14
		M	14	-	-	-	-	-	-	12
Leea indica (Burm.f.) Merr.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	12	-	-	-	-	-
Limonia acidissima L.	ND									
Murraya koenigii (L.)	Lf	A	-	-	-	-	-	-	-	-
Spreng		M	12	-	-	-	-	-	-	10
Sapendaceae										
Allophylus serratus (Roxb.) Kurz	ND									
Schleichera oleosa (Lour.)	Wp	A	-	-	-	-	-	-	-	-
Oken		M	-	-	-	-	-	-	-	10
Sapotaceae										
Mimusops elengi L	Bk	A	-	-	10	12	-	-	-	-
		M	-	-	14	12	12	-	12	-
Smilacaceae										
Smilax zeylanica L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	12	-	-	11	-	10	12
Solanaceae										
Nicotiana tabacum L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Sterculiaceae										
Helicteres isora L.	Lf	A	11	-	-	10	-	-	-	12
		M	-	-	-	8	-	-	-	10
	Fr	A	-	-	-	-	-	-	-	-
		M	11	-	-	-	-	-	-	12
	Rt	A	12	12	-	12	-	-	-	14
		M	14	10	-	10	-	10	-	12
Pterospermum acerifolium	Lf	A	10	10	-	-	-	10	-	12

(L.) Willd.		M	10	12	-	19	-	18	-	15
Strychnaceae										
Strychnos nux-vomica L.	Sd	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Tiliaceae										
Grewia hirsute Vahl.	Lf	A	-	-	-	-	-	-	-	-
		M	10	-	-	-	-	-	-	-
Grewia helicterifolia	Lf	A	-	-	-	-	-	-	-	-
Wall. ex. G. Don		M	-	-	-	-	-	-	-	-
Trapaceae										
Trapa natans L.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Ulmaceae										
Trema orientalis (L.) Bl.	Lf	A	-	-	-	-	-	-	-	-
		M	-	-	-	-	-	-	-	-
Verbenaceae										
Vitex negundo L.	Lf	A	10	10	-	-	-	-	-	-
		M	12	10	14	10	-	-	-	10
	Bk	A	10	-	10	-	-	-	-	12
		M	18	-	16	-	-	-	-	17
Zingiberaceae										
Curcuma angustifolia	Lf	A	-	-	-	-	-	-	-	-
Roxb.		M	-	-	8	-	-	-	-	10
Curcuma aromatica Salisb.	Rh	A	-	-	-	-	-	-	-	-
		M	-	-	12	-	-	-	-	12
Zingiber officinale Rosc.	Rh	A	-	-	-	-	-	-	-	-
		M	10	-	12	-	12	-	-	14

PU-Parts used, E-Extract, A-Aqueous, M-Methanol, Bl-bulb; Bk-bark; Fl-flower; Fr-fruit; Lf-leaf; Rt-root; Rh-rhizome; Rs-Resin; Sd-seeds; Tw-twig; Wp-whole plant, S1- *Escherichia coli*, S2- *Salmonella typhi*, S3- *Vibrio cholerae*, S4- *Vibrio alginolyticus*, S5- *Vibrio cholerae* 0139, S6- *Escherichia coli* O157:H7, S7- *Shigella dysentriae*, S8- *Salmonella typhimurium*

Among the test strains the most sensitive strains were recorded in decreasing order were *S. typhi* followed by *E. coli, Vibrio cholerae, S. typhimurium, V. alginolyticus, E. coli*

O157:H7 and *V. cholerae* O139. However, the strain *S. dysentriae* showed least activity in compared to above referred strains.

Nigella satavia (Ranunculaceae), used to treat numerous ailments including diarrhea and its essential oil has been shown to exhibit activity against *Staphylococcus aureus*, *Salmonella*, *Shigella*, *V. cholerae*, and *E. coli* (Ali et al. 2003). *Swertia corymbose* shows antibacterial activity against a wide range of microorganisms, including (*E. coli*, *Salmonella* sp., *V. cholerae* and *Staphylococcus aureus*) that cause diarrhea. Vijaya et al. 1997 reported 10 Indian traditional plants (*Allium sativum*, *Bauhinia racemosa*, *Camellia sinensis*, *Euphorbia hirta*, *Cissampelos pareira*, *Acorus calamus*, *Psidium guajava* and *Sphaeranthus indicus*) used to treat dysentery and diarrhea showed high antibacterial activity. Methanol and water extracts of a number of medicinal plants used to treat dysentery and diarrhea in the Democratic Republic of Congo showed activity against one or more enteropathogens, including *Shigella*, *Salmonella*, *E. coli*, *Vibrio* and *Campylobacter* (Longanga-Otshudi et al. 1999). Diehl et al. 2004 evaluated 60 traditionally used plants in human or veterinary medicine to treat worm infections (worms in general, round worms, Guinea worms, or flatworms), diarrhea, dysentery and abdominal pain.

Modern scientific evaluation of medicinal plants and herbs is concerned with validating the traditional use of plants as well as identifying the active components of extracts and preparations. With respect to traditional medicines used to treat diarrheal diseases, such medicines will continue to be used as long as there are communities with limited access to modern therapies. In future, it may be possible to supplement conventional ORS treatment with plant extracts resulting in complementary treatments that may lead to a reduction in the length of disease symptoms.

CONCLUSIONS

This study proves that use of plants for treatment of diarrhea and dysentery among the tribes of Similipal is still a major part of life and culture. The data collected show that majority of remedies are taken orally in fresh form. The study concludes that the tribes are depending on traditional medicinal uses and modern health system is far away from them. It is necessary to acquire and proper documentation of the knowledge of the tribes of Similipal, Mayurbahnj. The result obtained in the present study point out that in their crude form bout 75 % have scientifically proved as medicinal uses with respect to the antibacterial properties against diarrhea causing bacteria.

RECOMMENDATION

Surely, the evidence provided by this study will encourages further investigation in the expectation that alternative treatments for diarrheal diseases will be developed. Further

study is required to isolate compounds from plants which have not been reported as antimicrobial potential.

ACKNOWLEDGEMENT

The authors wish to thank Prof. S. K. Dutta and Dr. A. K. Bastia (North Orissa University) for their guidance during my Ph. D. and providing facilities for this work. We wish to express our profound gratitude to Dr. A. K. Biswal (North Orissa University) for identification of the plant samples. Thanks are also to S. K. Jena, B. Behera, D. Dubey, N. Patra and Y. K. Mohanta (North Orissa University), Dr. Sanat (Ayurvedic doctor, Badasahi, Mayurbahnj) for collection of plant samples and expert technical help during experiment. SKP is also grateful for financial support provided by DST, Govt. of Odisha. Finally, we wish to thank to all the tribal informants as they travelled with me and help in collection, identification and provided traditional uses of plants. We also thankful to all other informants those provide me information on medicinal uses and helps in communicating in vernacular languages. Hope that the information produced from these studies will be of use to them in future.

REFERENCES

Ahmad, I., Mehmood, Z. and Mohammad, F. 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol.* 62: 183-193.

Akindele, A.J. and Adeyemi, O.O. 2006. Evaluation of the antidiarrhoeal activity of *Byrsocarpus coccineus*. *J. Ethnopharmacol.* 108: 20-25.

Ali, B.H. and Blunden, G. 2003. Pharmacological and toxicological properties of Nigella sativa. Phytother Res. 17(4): 299-305.

Baldi, F., Bianco, M. A., Nardone, G., Pilotto, A. and Zamparo, E. 2009. Focus on acute diarrhoeal disease. *World J. Gastroenterol.* 15(27), 3341-3348.

Basile, A., Sorbo, S., Giordano, S., Ricciardi, L., Ferrara, S., Montesano, D., et al. 2000. Antibacterial and allelopathic activity of extract from *Castanea sativa* leaves. *Fitoterapia,* 71: 110-116.

Bauer A.W., Kirby, W.M.M., Sherris J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Path.* 45: 493-496.

Black, R.E. 1984. Diarrheal Diseases and Child Morbidity and Mortality. Population and Development Review: Child Survival: Strategies for Research, pp. 141-161.

Brijesh, S., Daswani, P.G., Tetali, P., Rojatkar, S.R., Antia, N.H., Birdi, T.J. 2006. Studies on *Pongania pinnata* (L) Pierre leaves: Understanding the mechanism(s) of action in infectious diarrhea. *J. Zhegiang University Science B* 7(8): 665-674.

Buret, A.G. 2007. Mechanisms of epithelial dysfunction in giardiasis. Gut, 56(3): 328-335.

Butler, K.M. 2008. Enterococcal infection in Children. *J. Seminars in Pediatric Infectious Diseases,* 17: 128-139

Casburn-Jones, A.C. and Farthing, M.J.D. 2004. Management of infectious diarrhoea. Gut, 53: 296-305.

Chacin-Bonilla, L. 2010. Epidemiology of *Cyclospora cayetanensis*: A review focusing in endemic areas. *Acta Tropica,* 115: 181-193.

Chaturongakul, S., Raengpradub, S., Wiedman, M. and Boor, K.J. 2008. Modulation of stress and virulence in Listeria monocytogenes. *Trend in Microbiology*, 16: 388-396.

Clarke, S.C. 2001. Diarrhoeagenic *Escherichia coli*- an emerging problem? *Diagnostic Microbiol Infectious Dis.* 41: 93-98.

Coutião Rodriguez R, Hernandez-Cruz P, Giles-Rãos H. 2001. Lectins in fruits having gastrointestinal activity: their participation in the hemagglutinating property of *Escherichia coli* O157:H7. *Arch. Med. Res.*, 32: 251-257.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*12: 564-582.

Cui, J., Wang, Z.Q. and Xu, B.L. 2011. Review: The epidemiology of human tirchinellosis in China during 2004-2009. *Acta Tropica*, 118: 1-5.

de Oliveira, C.E.V., Stamford, T.L.M., Neto, N.J.G., de Souza, E.L. 2010. Inhibition of *Staphylococcus aureus* in broth and meat broth using synergies of phenolics and organic acids. *Int. J. Food Microbiol.* 137: 312-316.

Dickman, K.G., Hempson, S.J., Anderson, J., Lippe, S., Zhao, L., Burakoff, R., et al. 2000. Rotavirus alters paracellular permeability and energy metabolism in Caco-2 cells. *Am. J. Physiology, Gastrointestinal and Liver Physiology,* 279(4): G757-G766.

Diehl, M.S., Kamanzi Atindehou, K., Tere, H. and Betschart, B. 2004. Prospect of anthelmintic plants in the Ivory Coast using ethnobotanical criteria. *J. Ethnopharmacol.* 95: 277-284.

Dupont, H.L. and Marshall, G.D. 1995. HIV-associated diarrhoea and wasting. Lancet 346: 352-356.

Epple, H.J., Schneider, T., Troeger, H., Kunkel, D., Allers, K., Moos, V., et al., 2009. Impairment of the intestinal barrier is evident in untreated but absent in suppressively treated HIV-infected patients. Gut, 58: 220-227.

Fabricant, D.S. and Farnsworth, N.R. 2001. The value of plants used in traditional medicine for drug discovery. *Env. Health Perspect.* 109: 69-75.

Farnsworth, N.R. 1966. Biological and phytochemical screening of plants. *J. Pharm. Sci.* 55(3): 225-269.

Farnsworth, N.R. 1988. Screening plants for new medicine. *In:* Wilson, E.O. (ed.), Biodiver-sity, National Academy Press, Washington, pp. 83-97.

Farnsworth, N.R. 1993. Biological approaches to the screening and evaluation of natural products. *In:* Rasoanaivo, P. and Ratsimamanga-Urverg, S. (eds), Biological evaluation of plants with reference to the Malagasy Flora, Madagascar, pp. 35-43.

Fernandez, M.I. and Sansonetti, P.J. 2003 *Shigella* interaction with intestinal epithelial cells determines the innate immune response in shigellosis. *Int. J. Med. Microbiol.* 293: 55-67.

Forgacs, I. and Patel, V. 2011. Diabetes and gastrointestinal tract. Medicine, 39 (5): 288-292.

From, C., Hormazabal, V., Granum, P.E. 2007. Food poisoning associated with pumilacidin-producing Bacillus pumilus in rice. *Int. J. Food Microbiol.* 115: 319-324.

Gale, G.A., Kirtikara, K., Pittayakhajounwut, P., Sivichai, S., Thebtaranonth, Y., Thongpanchang, C., Vichai, V., 2007. In search of cyclooxygenase inhibitors, anti-Mycobacterium tuberculosis and anti-malarial drugs from Thai flora and microbes. *Pharmacology and Therapeutics*, 115: 307-351.

Granum, E.P. 2006. Bacterial toxins as food poisons: Clinical, immunological aspects and applications of Bacterial protein toxins: The comprehensive sourcebook of Bacterial protein toxins (Ed. Alouf and Popoff) Pp. 949-958.

Grover JK, Khandar S, Vats V, Dhunnoo Y, Das D. 2002. Pharmacological studies on *Myristica fragrans*-antidiarrheal, hypnotic, analgesic and hemodynamic (blood pressure) parameters. *Meth. Find Exp. Clin. Pharmacol.* 10: 675-80.

Guerrant, R.L. 1998. Why America must care about tropical medicine: threats to global health and security from tropical infectious diseases. *Am. J. Tropical Med. Hygiene*, 59: 3-16.

Gutierrez, S.P., Sanchez, M.A.Z., Gonzalez, C.P., Garcia, L.A. 2007. Antidiarrhoeal activities of different plants used in traditional medicine. *Afr. J. Biotechnol.* 6(25), 2988-2994.

Henry-Stanley, M.J., Hess, D.J., Erickson, E.A., Garni, R.M. and Wells, R.M. 2003. Effect of Lipopolysaccharide on Virulence of Intestinal Candida albicans. *J. Surg. Res.* 113, 42-49.

Hodges, K. and Gill, R. 2010. Infectious diarrhea Cellular and molecular mechanisms. *Gut Microbes*, 1: 4-21.

Hu, L., Tall, B.D., Curtis, S.K., Kopecko, D.J. 2008. Enhanced microscopic definition of Campylobacter jejuni 81-176 adherence to, invasion of, translocation across, and exocytosis from polarized human intestinal Caco-2 cells. *Infection and Immunity*, 76(11): 5294-5304.

Huntington, H.P. 2000. Using traditional ecological knowledge in science: Methods and applications. *Ecol. Appl.* 10(5): 1270-1274.

Huycke, M.M., Moore, D.R. 2002. *In vivo* production of hydroxyl radical by enterococcus faecalis colonizing the intestinal tract using aromatic hydroxylation. *Free Rad. Biol. Med.* 33(6), 818-826.

Jeevan Ram, A., Bhakshu, L.M., Venkata Raju, R.R. 2004. *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. *J. Ethnopharmacol.* 90(2-3): 353-357.

Johns, T. 1996. *The Origins of Human Diet and Medicine: Chemical Ecology.* University of Arizona Press: Tucson.

Johns, T. 1999. The chemical ecology of human ingestive behaviors. *Ann. Rev. Anthrol.* 28: 27-50.

Kar, T., Mandal, K.K., Reddy, C.S. and Biswal, A.K. 2013. Ethnomedicinal plants used to cure diarrhoea, dysentery and cholera by some tribes of Mayurbhanj district, Odisha, India. *Life Sci. Leaflets, 2: 18-28*

Kenny, J.M. and Kelly, P. 2009. Protozoal gastrointestinal infections. *Medicine*, 39: 599-602.

Koff, R.S. 1992. Clinical manifestation and diagnosis of hepatitis A virus infection. *Vaccine*, 10: S15-S17.

Kone, W.M., Kamanzi-Atindehou, K., Terreaux, C., Hostettmann, K., Traore, D. and Dosso, M. 2004. Traditional medicine in North Cote-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *J. Ethnopharmacol*. 93: 43-49.

Koopmans, M. 2008. Progress in understanding norovirus epidemiology. *Current Opinion Infect. Dis.* 21, 544-552.

Krakauer, T., Li, B.Q., Young, H.A., 2001. The flavonoids baicalin inhibits superantigens-induced inflammatory cytokines and chemokines. *Federation of European Biochemical Societies Lett.* 500, 52-55.

Lauwaet, T., Oliveira, M.J., Callewaert, B., De Bruyne, G., Mareel, M., Leroy, A., 2004. Proteinase inhibitors TPCK and TLCK prevent *Entamoeba histolytica* induced disturbance of tight junctions and microvilli in enteric cell layers *in vitro*. *Int. J. Parasitol.* 34(7): 785-794.

Le Bouguenec, C. 2005. Adhesins and Invasins of pathogenic *Escherichia coli*. *Int. J. Med. Microbiol.* 295: 471-478.

Lengsfeld, C., Faller, G. and Hansel, A. 2007. Okra polysaccharides inhibit adhesion of Campylobacter jejuni to mucosa isolated from poultry in vitro but not *in vivo*. *Animal Feed Sc. Technol.* 135: 113-125.

Levine, J., Dykoski, R.K. and Janoff, E.N. 1995. Candida-Associated Diarrhea: A Syndrome in Search of Credibility. *Clin. Infect. Dis.* 21 (4): 881-886.

Lewis, W. and Elvin-Lewis, M. 2003. *Medical Botany: Plants Affecting Human Health.* John Wiley Interscience: New York.

Linscott, A.J. 2011. Food-borne illnesses. *Clin. Microbiol. Newsletter,* 33: 41-45.

Longanga Otshudi, A., Foriers, A., Vercruysse, A., Van Zeebroeck, A. and Lauwers, S. 1999. *In vitro* antimicrobial activity of six medicinal plants traditional used for the treatment of dysentery and diarrhea in Democratic Republic of Congo (DRC). *Phytomedicine,* 7: 167-172.

Lund, T., DeBuyser, M.L. and Granum, P.E., 2000. A new cytotoxin from Bacillus cereus that may cause necrotic enteritis. *Mol. Microbiol.* 38: 254-261.

Mansfield, L.S. and Gajadhar, A.A. 2004. *Cyclospora cayetanensis* a food and water-borne mcoccidian parasite. *Veterinary Parasitol.* 126: 73-90.

Mastroeni, P. and Maskell, D. 2006. *Salmonella* infections: Clinical, immunological, and molecular aspects. Cambridge University Press.

Mathabe, M.C., Nikolova, R.V., Lall, N. and Nyazema, N.Z. 2006. Antibacterial activities of medicinal plants used in the treatment of diarrhea disease in Limpopo Province, South Africa. *J. Ethnopharmacol.* 105: 286-293.

Mattison, K. 2010. Norovirus as a food-borne disease hazard. *Adv. Food Nutrition Res.* 62: 1-39.

Mbagwu, H.O. and Adeyemi, O.O. 2008. Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). *J. Ethnopharmacol.* 116: 16-20.

Mishra, N., Rout, S.D. and Panda, T. 2011. Ethno-zoological studies and medicinal values of Similipal Biosphere Reserve, Orissa, India. *Afr. J. Pharm. Pharmacol.* 5(1): 6-11.

Mitchell, T. 1998. Introduction: Host Interactions--Animals. In *Methods in Microbiology: Bacterial Pathogenesis* (Vol. 27), Williams P, Ketley J, Salmond G (eds). Academic Press: San Diego; 69-72.

Mohanta, R.K., Rout, S.D. and Sahu, H.K. 2006. Ethno medicinal plant resources of Similipal Biosphere reserve, Orissa, India. *Zoos Print J.* 21(8)/B: 2372-2374.

Monroe, S. and Polk, R. 2000. Antimicrobial use and bacterial resistance, *Curr. Opin. Microbiol*, 3(5): 496-501.

Munshi, M.H., Sack, D.A., Haider, K., Ahmed, Z.U., Rahman, M.M. and Morshed, M.G. 1987. Plasmid mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1, Lancet, 2: 419-421.

Navaneethan, U. and Giannella, R.A. 2011. Definition, Epidemiology, Pathophysiology, Clinical Classification and Differential Diagnosis of Diarrhea. *In:* Clinical Gastroenterology. G. Wu, ed. New York: Humana Press.

Ofek, I. and Sharon, N. 1990. Adhesins as lectins: Specificity and role in infection. In: K, Jann B (eds) Bacterial capsules and adhesins: Fact and Principles. *Current topic in Microbiol. Immunol.* Vol 151, Berlin: Springer, 91-113.

O-Hara, S.P. and Chen, X-M., 2011. The cell biology of cryptosporidium Cryptosporidium infection. *Microbes and Infection.*1-10.

Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., et al. 2004. Antiparasitic activities of medicinal plants used in Ivory Coast. *J. Ethnopharmacol*. 90: 91-97.

Padhi, L.P., Panda, S.K., Satapathy, S.N. and Dutta, S.K. 2011. *In vitro* evaluation of antibacterial potential of *Annona squamosa* L. and *Annona reticulata* L. from Similipal Biosphere Reserve, Orissa, India. *J. Agricultural Technol.* 7(1): 133-142.

Palombo E. 2006. Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: Modes of action and effects on intestinal function. *Phytother Res.* 20: 717-724.

Panda, S.K. and Dutta, S.K. 2011. Antibacterial activity from bark extracts of *Pterospermum acerifolium* (L.) Willd. *Int. J. Pharma. Sci. Res.* 584-595.

Panda, S.K., Dutta, S.K. and Bastia, A.K. 2010. Antibacterial activity of *croton roxburghii* Balak. against the enteric pathogens. *J. Adv. Pharma. Technol. Res.* 1(4): 388-391.

Panda, S.K., Rout, S.D., Mishra, N. and Panda, T. 2011a. Phytotherapy and traditional knowledge of tribal communities of Mayyurbhanj District, Orissa, India. *J. Phrmacognosy and Phytotherapy,* 3(7): 101-113.

Panda, S.K., Rout, S.D., Mishra, N. and Panda, T. 2011b. Folk uses of some medicinal plants by Kol tribes of Similipal Biosphere Reserve, Orissa, India. *Int. J. Biol. Technol.* 2(1): 16-20.

Panda, S.K., Padhi, L.P. and Mohanty, G. 2011c. Antibacterial activities and phytochemical analysis of *Cassia fistula* (Linn.) leaf. *J. Adv. Pharma. Technol. Res.* 2(1): 62-67.

Panda, S.K., Patro, N., Sahoo, G., Bastia, A.K. and Dutta, S.K. 2012. Anti-diarrheal activity of medicinal plants of Similipal Biosphere Reserve, Odisha, India. *Int. J. Med. Aromatic Plants,* 1 (2): 123-134.

Panda, S.K. 2014. Ethnomedicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. *J. Ethnopharmacol.* 151: 158-175.

Panda, S.K., Bastia, A.K. and Sahoo, G. 2014. Process characteristics and nutritional evaluation of *handia* - A cereal based ethnic fermented food from Odisha. *Ind. J. Tradit. Knowl.* 13 (1): 149-156.

Pandey, A.K. and Rout, S.D. 2002. Medicinal plants of Similpal Biosphere Reserve-Prospectives of Plant Biodiversity. (Eds Das AP); Bishen Singh Mahendra Pal Singh, Dehra.

Pandey, A.K. and Rout, S.D. 2006. Ethnobotanical uses of plants by tribals of Similipal Biosphere Reserve (Orissa), pp. 681-696.

Pandey, A.K., Varma, S.K., Pandit, N. and Singh, L.A.K. 2000. Medicinal Plants of Similipal Biosphere Reserve. *J. Ind. Bot. Soc.* 79: 52-53.

Parekh, J., Nair, R. and Chanda, S. 2005. Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Ind. J. Pharmacol.* 37: 408-409.

Peirano, G., Souza, F.S. and Rodrigues, D.P. 2006 Frequency of serovers and antimicrobial resistance in *Shigella* spp., *Mem Inst Oswaldo Cruz Rio de Janeiro*, 101(3): 245-250.

Perez-Bosque, A. and Moreto, M. 2010. A rat model of mild intestinal inflammation induced by Staphylococcus aureus enterotoxins B. The 3rd International Immunonutrition Workshop held in Spain on 21-24 Oct., 2009. *Proceedings of the Nutrition Society*, 69: 447-453.

Perumal Samy, R., Ignacimuthu, R. and Raja, D.P. 1998. Screening of 34 Indian medicinal plants for antibacterial properties. *J. Ethnopharmacol.* 62(2): 173-182.

Perumal Samy, R., Ignacimuthu, R. and Sen, A. 1999. Preliminary screening of ethno-medicinal plants from India. *J. Ethnopharmacol.* 66(2): 235-240.

Podewils, L.J., Mintz, E.D., Nataro, J.P., Parashar, U.D. 2004. Acute, infectious diarrhoea among Children in Developing Countries. *J. Seminars in Pediatric Infectious Diseases,* 155-168.

Prashanthkumar, V., Chauhan, N.S., Haris, P. and Rajani, M. 2006. Search for antibacterial and antifungal agents from selected Indian medicinal plants. *J. Ethnopharmacol.* 107(2): 182-188.

Rabbani GH, Teka T, Saha SK, Zaman B, Majid N, Khatun M, *et al.* 2004. Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea. *Digestive Disorders Sci.* 49: 475-484.

Ralstron, K.S., Petri Jr., W.A. 2011. Tissue destruction and invasion by *Entamoeba histolytica*. *Trends in Parasitology*, 27, 253-262.

Ramesh, N., Viswanathan, M.B., Saraswathy, A., Balakrishna, K., Brindha, P. and Perumalsamy, L. 2002. Antimicrobial and phytochemical studies of *Swertia corymbosa. Fitoterapia,* 73: 160-164.

Ravikumara, M. 2008. Investigation of chronic diarrhoea. *Paediatrics and Child health,* 18(10): 441-447.

Robert, K., Egon, S., Daneila, B., Florian, D., Christoph, W., Gunter, J.K. and Emil, C.R. 2001. Role of *Candida* in antibiotics associated diarrhea. *J. Infect. Dis.* 184: 1065-1069.

Rosengren, Å., Fabricius, A., Guss, B., Sylvén S., Lindqvist, R., 2010. Occurrence of foodborne pathogens and characterization of Staphylococcus aureus in cheese produced on farm-dairies. International journal of Food microbiology

Rout S.D. and Panda S.K. 2010 Ethno medicinal plant resources of Mayurbhanj district, Orissa. *Ind. J. Tradit. Knowl.* 9 (1): 68-72.

Rout, S.D. and Pandey, A.K. 2007. Ethnomedicobiology of Similipal Biosphere Reserve, Orissa. In:Das AP, Pandey AK (Eds). *Advances in Ethnobotany*. Dehera Dun, pp. 247-252.

Rout, S.D. and Thatoi, H.N. 2009. Ethnomedicinal practices of Kol tribes in Similipal Biosphere Reserve, Orissa, India. *Ethnobotanical Leaflets,* 13: 379-87.

Rout, S.D., Panda, T. and Mishra, N. 2009. Ethnobotanical studies of Similpal Tiger Reserve. *Ethnobotany*, 21(1&2): 80-83.

Sagar, L., Sehgal, R. and Ojha, S. 2005. Evaluation of antimotility effect of *Lantana camara* L. var. acuelata constituents on neostigmine induced gastrointestinal transit in mice. *BMC Compl. Alter. Med.* 175: 18.

Sairam, K., Hemalatha, S., Kumar, A., Srinivasan, T., Ganesh, J., *et al.* 2003. Evaluation of antidiarrhoeal activity in seed extracts of *Mangifera indica. J. Ethnopharmacol.* 84: 11-15.

Sanchez, J. and Holmgren, J. 2005. Virulence factors, pathogenesis and vaccine protection in cholera and ETEC diarrhea. *Curr Opin Immunol.* 17: 388-398.

Schiller, L.R. 1999. Secretory diarrhea. *Current Gastroenterology Reports*, 1: 389-397.

Schmitz, H., Rokos, K., Florian, P., Gitter, A.H., Fromm, M., Scholz, P., et al., 2002. Supernatants of HIV-infected immune cells affect the barrier function of human HT-29/B6 intestinal epithelial cells. *AIDS*, 16(7): 983-991.

Schulzke, J.D., Troger, H., Amasheh, M. 2009. Disorders of intestinal secretion and absorption. *Best Practice and Res. Clin. Gastroenterol.* 23, 395-406.

Shaphiullah, M., Bachar, S.C., Kundu, J.K., Begum, F., Uddin, M.A., Roy, S.C., *et al.* 2003. Antidiarrheal activity of the methanol extract of *Ludwigia hyssopifolia* L. *Pak. J. Pharma Sci.*16: 7-11.

Shilpi, J.A., Taufiq-Ur-Rahman, M., Uddin, S.J., Alam, M.S., Sadhu, S.K. and Seidel, V. 2006. Preliminary pharmacological screening of *Bixa orellana* L. leaves. *J. Ethnopharmacol.* 108: 264-271.

Soderholm, J.D. and Perdue M.H. 2006. Effect of stress on intestinal mucosal function. Physiology of the gastrointestinal tract, Fourth Edition, edited by Leonard R. Johnson. Academic Press.

Sood, A. and Pacheco, P.A. 2002. Infectious diarrhea. *Current Treatment Options in Gastroenterol.* 4: 211-216.

Spiller, R.C. 2004. Inflammation as a basis for functional GI disorder. *Best Practice Res. Clin. Gastroenterol.* 18: 641-661.

Sun, J., Hu, X.-L., Le, G.-W. and Shi, Y.-L. 2010. *Lactobacilli* prevent hydroxy radical production and inhibit *Escherichia coli* and *Enterococcus* growth in system mimicking colon fermentation. *Lett. Appl. Microbiol.* 50: 264-269.

Sur, D., Ramamurthy, T., Deen, J. and Bhattacharya, S.K. 2004. Shigellosis: Challenges and management issues, *Indian J. Med. Res.* 120(5): 454-462.

Teke, G.N., Kuiate, J.R., Ngouateu, O.B. and Gatsing, D. 2007. Antidiarrhoeal and antimicrobial activities of Emilia coccinea (Sims) G. Don extracts. *J. Ethnopharmacol.* 112: 278-283.

Thakurta, P., Bhowmik, P., Mukherjee, S., Hajra, T., Patra, A. and Bag P. 2007. Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India. *J. Ethnopharmacol.* 111: 607-612.

Todd, E.C.D. and Notermans, S. 2011. Surveillance of listeriosis and its causative pathogen, Listeria monocytogenes. *Food Control,* 22: 1484-1490.

Troeger, H., Epple, H.J., Schneider, T., Wahnschaffe, U., Ullrich, R., Burchard, G.D., et al., 2007. Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. *Gut*, 56(3): 328-335.

Valsaraj, R., Pushpangadan, P. Smitt, U.W., Adsersen, A. and Nyman, U. 1997. Antimicro-bial screening of selected medicinal plants from India. *J. Ethnopharmacol.* 58(2): 75-83.

Van Wyk, B. and Wink, M. 2004. Health disorders and Medicinal plants. In: Medicinal plants of the world. Published by Briza Publications CK90/11690/23, P O Box 56569, Arcadia 0007, Pretoria, South Africa, Pp 351-370.

Vijaya, K. and Ananthan, S.J. 1997. Microbiological screening of Indian medicinal plants with special reference to enteropathogens. *Alter. Complem. Med.* 3: 13-20.

Vitali, F., Fonte, G., Saija, A. and Tita, B. 2006. Inhibition of intestinal motility and secretion by extracts of *Epilobium* spp. in mice. *J. Ethnopharmacol.* 107: 342-348.

Vlietinck, A.J. and VandenBerghe, D.A. 1991. Can ethnopharmacology contribute to the development of antiviral drugs? *J. Ethnopharmacol.* 32: 141-153.

www.who.int/.../NCMH_Burden%20of%20disease_(29%20Sep%202005). NCMH Background Papers · Burden of Disease in India; Estimation of the burden of diarrheal diseases in India.

Wynn, S.G. and Fougere, B.J. 2007. A systems-based approach: Diarrhea-Therapeutic rationale. In: Veterinary Herbal medicine. (Edited by Wynn GS and Fougere BJ); Elsevier 11830 Westline Industrial Drive, St Louis, Mussolini 63146: 33336-337.

CHAPTER EIGHT

MAXIMUM LIKELIHOOD ESTIMATES IN RELIABILITY OF TYPHOID FEVER DRUG IN THE RECOVERY OF TYPHOID FEVER PATIENTS AT AKUSE (A CASE STUDY AT THE AKUSE GOVERNMENT HOSPITAL)

Eric Boahen
Department of Statistics, Faculty of Mathematical Sciences, University For Development Studies –Tamale.Box 24 uds Navrongo- Ghana.

ABSTRACT

Typhoid fever is one of the top ten outpatient diseases that is recorded by the Akuse Government Hospital. Akuse is a big town located in the eastern region of Ghana. The Government Hospital located in the area recorded a continuous substantial increase in the number of typhoid fever cases. This substantial increase was partly attributed to the fact that the probability of an individual to be healed of the disease was relatively low. As a result of this, a five staged clinical trial was conducted by the hospital to assess the probability of being cured after receiving treatments. This five staged clinical trial was used in the research to assess the survivability growth and reliability of the typhoid fever drugs administered. The research was carried out by first, obtaining the observed survival probabilities at each stage and then the maximum likelihood estimates and least squares estimates were found. The Newton-Raphson procedure was used in the calculation of the maximum likelihood estimates. An appropriate (1-a) 100 percent lower confidence limit was then constructed for each stage of the clinical trial. In clinical trials, patients response to treatment is classified as success or failure, therefore, the overall probability of success in the clinical trial was then assessed with the aid of smoothen constants. The study identified that the drugs administered had huge effect on the survival of an individual and it was therefore recommended that the Hospital creates an awareness program for the people of Akuse to know that treating typhoid the drugs used in the clinical trial helps in curing typhoid. Also, the Akuse Government Hospital in their endeavor to the increase the curing rate of typhoid fever in the township should encourage patients to take drugs prescribed to the patients well.

INTRODUCTION

Introduction

The purpose of the study was to find out the reliability and growth in the healing process of typhoid fever at Akuse in the Lower Manya District. This chapter consists of

background of the study, problem statement, purpose of the study, objectives, research questions, scope of the study and Limitations.

Background of the study

Akuse is a town located in the Eastern Region of Ghana off the Akosombo – Tema highway. Akuse is under the Lower Manya Constituency and is situated close to the Volta River. Akuse Government Hospital is located at the entrance of the town, opposite the Ghana Commercial Bank. The hospital serves people from other surrounding towns such as Somanya, Atua, Kpong among other smaller towns located closes to Akuse.

Typhoid Fever also known as Enteric Fever can be a life – threatening febrile disease, caused by the bacterium *Salmonella Typhi*. According to the Centre for Disease and Control (CDC) an estimated 22 million cases of typhoid fever and 200 000 related deaths occur worldwide every year. Typhoid is common in most parts of the developing world. In Ghana, a study conducted in 2010 has shown that typhoid fever ranks among the leading causes of outpatient illness accounting for 0.92% of hospital admissions.

The disease is most common in developing countries of which Ghana is no exception contributing factors being poor sanitary system and lack of antibiotics putting travelers to Asia America and Africa in high risk group.

Typhoid fever belongs to the family Enterobacteriaceae and has been associated with gastroenteritis and food borne diseases. The salmonella species is the etiological agents of most food borne disease and gastroenteritis.

In the year 2011, three hundred and twenty two cases (322) of typhoid fever were recorded in the Akuse Government Hospital with seven hundred and seventy two (772) cases and one thousand (1000) cases in 2012 and 2013 respectively.Domestically acquired typhoid fever, however, is often transmitted by chronic carrier of salmonella typhi. After recovering from typoid fever, some individuals carry the bacterium asymptomatically for long periods; others are asymptomatic carriers without ever having the disease. The silent carriers contribute to continued episodes of infection. Most of the disease is acquired by people travelling overseas (Prescott, 1999).

The disease, typhoid fever, is caused by Salmonella typhi which is acquired by ingestion of food or water contaminated by faeces of infected humans. The pathogen colonizes the small intestines penetrating the lymph node spleen and other lymphoid tissues within 6-14 days after exposure, to headache and fever developments. The latter can continue for weeks and rise about 40^0 Celsius. In most cases, salmonella typhi is

shed in faeces for several weeks. However, approximately 3% of those who recover continue to shed salmonella typhi for extended periods but show no symptoms of the disease. In such individuals, termed carriers, the bacterium grows in the gall bladder and finds its way to the intestines through the bile duct.

Typhoid fever is usually confirmed by identifying Salmonella Typhi in a culture of the blood or other body fluid (urine and stools). For the culture, a small sample of the patient's blood, urine and stool is placed in a container to growth the bacteria. This takes between 48 to 72 hours, for the culture to be checked under a microscope for the presence of the bacteria.

According to CDC, one can get typhoid fever if food or drink beverages that have been handled by a person who is shedding *Salmonella* Typhi are ingested or if sewage contaminated with *Salmonella* Typhi bacteria gets into the water used for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where hand washing is less frequent and water is likely to be contaminated with sewage. World Health Organization (WHO) also mentioned the mode of transmission of the disease as through the ingestion of food or drink contaminated by the faeces or urine of infected persons.

Typhoid fever in Akuse is mostly attributed to the works of witchcraft and other demonic powers. The people of Akuse believe that the disease can be transmitted onto a person by spiritual means. In a blog posted on March 10th, 2012 by one researcher named BiorKwerBiorJr (Borglobe), he mentions that the people of South Sudan and other neighboring countries have a myth that typhoid fever is attributed to the sun.

Case-fatality rates of 10% can be reduced to less than 1% with appropriate antibiotic therapy. However, strains resistant to chloramphenicol and other recommended antibiotics (ampicillin, cotrimoxazole and even ciprofloxacin) have become prevalent in several areas of the world. It usually takes two weeks for patients treating typhoid fever to be fully cured.

Typhoid fever can also be treated herbally using the roots, bark and leaves of plants. The use of mauringa is highly recognized in Akuse for the treatment of typhoid fever and other fever related diseases.

CDC described measures of preventing the disease in four ways; boil it, cook it, peel it or forget it. The Centre for Disease Control listed these measures of preventing or avoiding typhoid;

- If you drink water, buy it bottled or bring it to a rolling boil for 1 minute before you drink it. Bottled carbonated water is safer than uncarbonated water.
- Ask for drinks without ice unless the ice is made from bottled or boiled water. Avoid popsicles and flavored ices that may have been made with contaminated water.
- Eat foods that have been thoroughly cooked and that are still hot and steaming.
- Avoid raw vegetables and fruits that cannot be peeled. Vegetables like lettuce are easily contaminated and are very hard to wash well.
- When you eat raw fruit or vegetables that can be peeled, peel them yourself. (Wash your hands with soap first). Do not eat the peelings.

The following problems are faced in the treatment process of typhoid fever;

- Patients do not take medications as prescribed by the doctor or health practitioner.
- Patients do not heed to the doctor's advice by not returning for a checkup after two weeks when the bacteria and the symptoms are expected to have gone.
- Some patients feel reluctant to report signs and symptoms early but rather wait till the symptoms become severe before seeking medical attention

Problem Statement

Typhoid fever is one of the leading causes of death among children and adults in Ghana.

Study has shown that, EasternRegion has high cases of typhoid. Our study has also shown that, Eastern Region is one of the regions with high typhoid cases due to the open defecation practice, dirty surroundings and unhygienic practices.Typhoid fever is one of the top 10 out-patient diseases in Ghana according to the Ghana health service. It is rated among the top killer diseases in Ghana.

Typhoid fever cases in Akuse are on the rise. The typhoid fever cases at Akuse in the eastern region for the year 2011, 2012 and 2013 are three hundred and twenty two (322), seven hundred and seventy two (772) and one thousand (1000) respectively and the recovery rate for the patients have been low. There is a situation at Akuse where infected persons who report to the hospital, diagnosed with the disease, given medication to follow and are asked to come back for review end up, taking the medication until when the infected person feels his situation is better but not when the dosage of the medication has been fully completed.

The infected persons also ignore the review instruction set by the doctor or health practitioner. Therefore, the fact that, an infected person is fully cured of the disease and

the bacteria have been removed from the system is in doubt. These patients will still harbor the bacteria in their bodies.When these infected persons have a bowel movement, they may pass stools (faeces) that contain the Salmonella typhi bacteria.

There is a low level of sanitation at Akuse and hygienic conditions are also poor. Infected persons, who do not wash their hands before handling food and water, contaminate the food. When another person consumes tainted food or water, he picks up the typhoid bacteria and become infected.

Some people, known as chronic carriers, still harbor typhoid bacteria (and can still contaminate food and water supplies) even after receiving antibiotic treatment and proving to be free of symptoms.

All these conditions add up to the increasing rate of typhoid cases at Akuse. In this regard, the research was interested in studying the survivability growth and the reliability of typhoid fever drugs at Akuse in the Eastern Region of Ghana.

Purpose of the study

The purpose of the study is to:

- Assess the survivability growth rate of the healing process
- Assess the reliability of the drug

Objectives of the study

The main objectives of the study are to assess the reliability of the drug and growth in the healing process of typhoid fever and also to determine the overall probability of success or failure in each stage of the clinical trial.

Research Questions

These research questions have been outlined for the project.

- How reliable is the drug?
- Is the healing process improving?
- Is the overall survival probability at each stage of the clinical trial good?

Scope of the study

The study is confined to the Lower Manya District of the Eastern Region, Akuse to be precise. The data used for the project was collected in five stages with ten patients in each stage. The conclusion of the study was however generalized and made applicable to all typhoid cases treated with cefuroxime and ciprofloaxin throughout the country. The study was not made applicable to other drugs used in the treatment of typhoid fever.

Limitations of the Study

These constraints among others were encountered during the study;

- Those in charge of the data were reluctant to give out the data for analysis.
- It was difficult for those at the records department to really understand the kind of data needed for the research.
- It was not easy moving from Navrongo to Akuse to collect the data as far as monetary issues are concerned.

METHODOLOGY

Introduction

This chapter introduces the methodology adopted in the study. It discusses the study area, the design of the research, sample size selection, population of study, technique used in the data collection, sampling techniques, statistical analysis, and procedures and describes the theory of the model used, formulations and methods of analyzing the available data to satisfy the objectives of the study.

Study area

The study was conducted among typhoid fever patients in the Akuse Government Hospital. The hospital is one of the biggest in the ManyaKrobo District and also one of the oldest in the country. The Akuse Government hospital is located at the entrance of the Akuse town, opposite the Ghana Commercial Bank. It serves people from neighboring towns and villages. The people of Akuse are mainly farmers and petty traders. Some of the indigenes are employed with the anana farm plantation located close to the town. The Kpong Generation Station, with four power turbines, which produces power for the national grid is also located at Akuse. Therefore, Akuse also inhabits the Volta River Authority (VRA) workers, who work in the generation station.

Population and sample

The population of the study was made up of typhoid fever patients in the ManyaKrobo district. However, the accessible population of the research consisted of all typhoid fever patients who were diagnosed with the disease in the month of October. A total number of 63 cases was recorded by the hospital for that month.

Sources of data collected

Secondary data was our main source of collecting data for the study. The secondary data was collected from the hospital in a clinical trial conducted in five stages. The trial was to test the survivability growth of patients' remission from typhoid fever. The trial had each in each stage ten patients who were receiving treatment from typhoid. Each patient had a healing period of two weeks since the typhoid fever drugs used are supposed to cure a patient with the disease within that period. Each stage of the clinical trial has a new group of patients thereby making the trial carried out, independent.

Statistical analysis, procedures and models used

The data collected was a research conducted in 5 (five) stages such that at the *ith*stage $n_i$patients enter the study each with a probability p_i of surviving the *ith* stage. At each stage a new group of patients is entered; thus stages are assumed to be independent as well as identical in their time periods. After the *ith* stage, the probability there are $x_i$survivors (number of patients healed) is

$$pr\{X = x\} = \binom{n_i}{x_i} p_i^x (1 - p_i)^{n_i - x_i} \quad \ldots(1)$$

Some general parametric growth models is assumed by $p_i$for measuring survivability growth from stage to stage. Examples of these growth models are the hyperbolic growth model, exponential growth model and the logistic growth model.

The proportion of survivors from the data, increases from stage to stage. $p_i$is a function of two parameters as well as the stage number *i*. The exponential model is given by

$$p_i = 1 - \alpha_1 exp(-\alpha_2 i) \quad \ldots(2)$$

where$0 < \alpha_1 < e^{\alpha_2}, \alpha_2 > 0$ are the parameters of the model, $i = 1,2,3,4, \ldots, k$. Two methods commonly used to estimate α_1 and α_2 are least squares and maximum likelihood. Maximum likelihood estimators are generally preferred to least squares estimators because of the desirability of large sample properties of maximum likelihood estimator. On the other hand least square estimators are often obtainable in closed form and are a good first approximation to the maximum likelihood estimator.

Least Squares

Let us define $\psi(\alpha_1, \alpha_2)$ as

$$\psi(\alpha_1, \alpha_2) \equiv \sum_{i=1}^{k} \left[\frac{x_i}{n_i} - p_i(\alpha_1, \alpha_2)\right]^2 \quad \ldots(3)$$

The least squares estimator α_1^* and α_2^* are the values of the parameters $\alpha_1$and α_2, respectively, that simultaneously minimize $\psi(\alpha_1, \alpha_2)$. Assuming that $p_i(\alpha_1, \alpha_2)$ is differentiable in $\alpha_1$and α_2, $i = 1,2, \ldots, k$, and that the minimum is obtained by differentiation, we find α_1^* and α_2^*as the solution of

$$\sum_{i=1}^{k} \left[\frac{x_i}{n_i} - p_i(\alpha_1, \alpha_2)\right]^2 \frac{\partial p_i(\alpha_1^*, \alpha_2^*)}{\partial \alpha_1^*} = 0 \quad \ldots(4)$$

and

$$\sum_{i=1}^{k} \left[\frac{x_i}{n_i} - p_i(\alpha_1^*, \alpha_2^*)\right]^2 \frac{\partial p_i(\alpha_1^*, \alpha_2^*)}{\alpha_2^*} = 0 \quad \ldots(5)$$

where

$$\frac{\partial p_i(\alpha_1^*,\alpha_2^*)}{\partial \alpha_j^*} \equiv \left.\frac{\partial p_i(\alpha_1,\alpha_2)}{\partial \alpha_j}\right|_{\substack{\alpha_1=\alpha_1^* \\ \alpha_2=\alpha_2^*}} \quad j = 1,2. \qquad \ldots(6)$$

That is, α_1^* and α_2^* are the least squares estimators ofα_1 andα_2, respectively.

Maximum Likelihood

Given the probability that x_i patients of n_i on trial at stage i survive, $i = 1,2,\ldots,k$.Thus the k stages being statistically independent, the likelihood function for all k stages is given by

$$L(\alpha_1,\alpha_2) = \prod_{i=1}^{k} \binom{n_i}{x_i} [p_i(\alpha_1,\alpha_2)]^{x_i}[1 - p_i(\alpha_1,\alpha_2)]^{n_i - x_i} \qquad (7)$$

Assuming that the parameter vector (α_1,α_2) can be maximized with respect to the observations by maximum likelihood procedures, the maximum likelihood estimator $\hat{\alpha}_1$ and $\hat{\alpha}_2$ are the values of the parameters $\alpha_1$and α_2, respectively, that simultaneously maximize $L(\alpha_1,\alpha_2)$ or equivalently $log_e L(\alpha_1,\alpha_2)$. The vector $(\hat{\alpha}_1,\hat{\alpha}_2)$ is then the simultaneous solution of

$$\sum_{i=1}^{k} \frac{x_i}{p_i(\hat{\alpha}_1,\hat{\alpha}_2)} \frac{\partial p_i(\hat{\alpha}_1,\hat{\alpha}_2)}{\partial \hat{\alpha}_1} - \sum_{i=1}^{k} \frac{n_i - x_i}{1-p_i(\hat{\alpha}_1,\hat{\alpha}_2)} \frac{\partial p_i(\hat{\alpha}_1,\hat{\alpha}_2)}{\partial \hat{\alpha}_1} = 0 \qquad (8)$$

and

$$\sum_{i=1}^{k} \frac{x_i}{p_i(\hat{\alpha}_1,\hat{\alpha}_2)} \frac{\partial p_i(\hat{\alpha}_1,\hat{\alpha}_2)}{\partial \hat{\alpha}_2} - \sum_{i=1}^{k} \frac{n_i - x_i}{1-p_i(\hat{\alpha}_1,\hat{\alpha}_2)} \frac{\partial p_i(\hat{\alpha}_1,\hat{\alpha}_2)}{\partial \hat{\alpha}_2} = 0 \qquad (9)$$

that maximizes $L(\hat{\alpha}_1,\hat{\alpha}_2)$, where

$$\frac{\partial p_i(\hat{\alpha}_1,\hat{\alpha}_2)}{\partial \hat{\alpha}_j} \equiv \left.\frac{\partial p_i(\alpha_1,\alpha_2)}{\partial \alpha_j}\right|_{\substack{\alpha_1=\hat{\alpha}_1 \\ \alpha_2=\hat{\alpha}_2}} \quad j = 1,2. \qquad (10)$$

Estimation for the Exponential Growth Model

The exponential model was chosen for this research after plotting the survival probabilities at each stage of the trial.

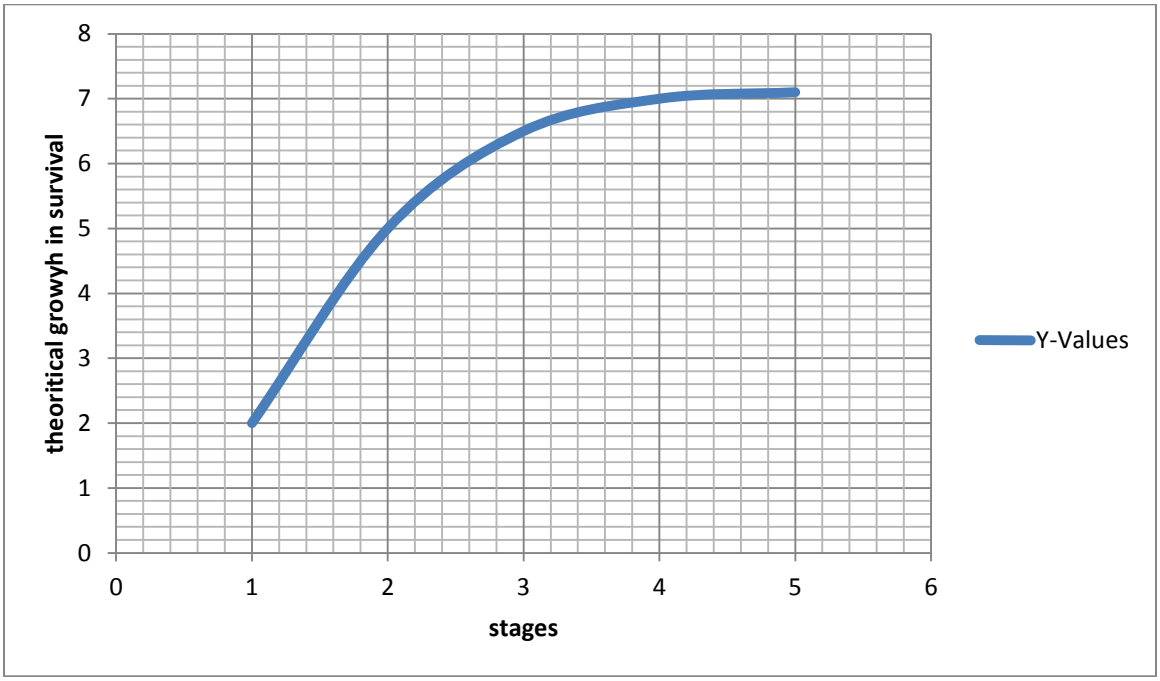


Fig 1: Plot of survival probabilities against stages

This model depicts a slower growth in the early stages which sustains itself in the later stages.

Let $p_i(\alpha_1, \alpha_2)$ be defined by the exponential growth model. That is

$$p_i(\alpha_1, \alpha_2) = 1 - \alpha_1 exp(-\alpha_2 i), \qquad i = 1,2, \dots, k. \qquad (11)$$

Since for this model

$$1 - p_i(\alpha_1, \alpha_2) = \alpha_1 exp(-\alpha_2 i), \qquad i = 1,2, \dots, k, \qquad (12)$$

the least squares estimator α_1^* and α_2^* are obtained in the following ways. First

$$E\left(\frac{n_i - x_i}{n_i}\right) = \alpha_1 exp(-\alpha_2 i), \qquad i = 1,2, \dots, k, \qquad (13)$$

Where $x_i \leq n_i - 1$.

As a result of this a least squares fit on the logarithm is used to obtain $\alpha_1^*$and α_2^*. The least squares equation$*$ is then

$$\psi(\alpha_1, \alpha_2) = \sum_{i=1}^{k} \left[log_e \frac{n_i - x_i}{n_i + 1} - log_e \alpha_1 + \alpha_2 i\right]^2. \qquad (14)$$

Let us set $z_i \equiv log_e\{(n_i - x_i)/(n_i - 1)\}$. Differentiating $\psi(\alpha_1, \alpha_2)$ with respect to α_1 and α_2 and setting the resulting equations equal to zero we obtain

$$\sum_{i=1}^{k} iz_i - klog_e\alpha_1^* + \frac{k(k+1)}{2}\alpha_2^* = 0 \quad (15)$$

and

$$\sum_{i=1}^{k} iz_i - \frac{k(k+1)}{2}log_e\alpha_1^* + \frac{k(k+1)(2k+1)}{6}\alpha_2^* = 0. \quad (16)$$

Solving in terms of $log_e\alpha_1^*$ and α_2^* we find

$$log_e\alpha_1^* = 2[k(k-1)]^{-1}\{(2k+1)\sum_{i=1}^{k} z_i - 3\sum_{i=1}^{k} iz_i\} \quad (17)$$

and

$$\alpha_2^* = 6[k(k-1)]^{-1}\left\{\sum_{i=1}^{k} z_i - 2\frac{\sum_{i=1}^{k} iz_i}{k+1}\right\} \quad (18)$$

These are used as the least squares estimators of the exponential growth model. The maximum likelihood function for the exponential growth model. Let $L(\alpha_1, \alpha_2)$ be the likelihood function for all k stages of the trials.

Then we have

$$L(\alpha_1, \alpha_2) = \prod_{i=1}^{k}\binom{n_i}{x_i}\left(1 - \alpha_1 exp(-\alpha_2 i)\right)^{x_i}\left(\alpha_1 exp(-\alpha_2 i)\right)^{n_i - x_i} \quad (19)$$

Taking logarithms and then differentiating with respect to $\alpha_1$and α_2, the maximum likelihood estimators $\hat{\alpha}_1$ and $\hat{\alpha}_2$ are obtained as the unique solution to

$$-\sum_{i=1}^{k}\frac{x_i}{[exp(\hat{\alpha}_2 i) - \hat{\alpha}_1]} + \frac{\sum_{i=1}^{k}(n_i - x_i)}{\hat{\alpha}_1} = 0 \quad (20)$$

And

$$\hat{\alpha}_1\sum_{i=1}^{k}\frac{ix_i}{[exp(\alpha_2 i) - \hat{\alpha}_1]} - \sum_{i=1}^{k} i(n_i - x_i) = 0. \quad (21)$$

Therefore taking the logarithm and differentiating with respect to $\alpha_1$and α_2, we have

$$\frac{\partial^2 log_e L}{\partial\alpha_1^2} = -\left[\sum_{i=1}^{k}\frac{x_i}{[exp(\alpha_2 i) - \alpha_1]^2} + \sum_{i=1}^{k}\frac{n_i - x_i}{\alpha_1^2}\right], \quad (22)$$

$$、\frac{\partial^2 log_e L}{\partial\alpha_1\partial\alpha_2} = \sum_{i=1}^{k}\frac{ix_i exp(\alpha_2 i)}{[exp(\alpha_2 i) - \alpha_1]^2}, \quad (23)$$

and

$$\frac{\partial^2 log_e L}{\partial\alpha_2^2} = -\alpha_1\sum_{i=1}^{k}\frac{i^2 x_i exp(\alpha_2 i)}{[exp(\alpha_2 i) - \alpha_1]^2} \quad (24)$$

Confidence regions and intervals for the exponential growth model

An approximate (1-α)100 percent elliptical confidence region for the parameter vector $\binom{\alpha_1}{\alpha_2}$ is

$$\sigma^{11}(\hat{\alpha}_1 - \alpha_1)^2 + \sigma^{22}(\hat{\alpha}_2 - \alpha_2)^2 + 2\sigma^{12}(\hat{\alpha}_1 - \alpha_1)(\hat{\alpha}_2 - \alpha_2) = \chi^2(1-\alpha;2) \quad \ldots(25)$$

Where $\chi^2(1-\alpha;2)$ is the (1-α)100 percentage point of the chi-square distribution with 2 degree of freedom and

$$\sigma^{ij} = -E\left[\frac{\partial^2 log_e L}{\partial\alpha_i \partial\alpha_j}\right], \qquad i,j = 1,2. \qquad (26)$$

For this model

$$\sigma^{11} = \alpha_1^{-1}\sum_{i=1}^{k}\frac{n_i}{exp(\alpha_2 i)-\alpha_1}, \qquad (27)$$

$$\sigma^{12} = -\sum_{i=1}^{k}\frac{in_i}{exp(\alpha_2 i)-\alpha_1}, \qquad (28)$$

and

$$\sigma^{22} = \alpha_1\sum_{i=1}^{k}\frac{i^2 n_i}{exp(\alpha_2 i)-\alpha_1}. \qquad (29)$$

We obtain an appropriate $(1-\alpha)100$ percent lower confidence limit $p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2)$ for the exponential growth model. We have

$$p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2) = (1 - \hat{\alpha}_1 \exp(-\hat{\alpha}_2 i)) - Z(1-\alpha)\sqrt{V\hat{a}r(1 - \hat{\alpha}_1 \exp(-\hat{\alpha}_2 i))}, \qquad (30)$$

Where

i. $\mathrm{Var}(1 - \hat{\alpha}_1 \exp(-\hat{\alpha}_2 i))$ is given as

$$Var(1 - \hat{\alpha}_1 \exp(-\hat{\alpha}_2 i)) = \exp(-2\alpha_2 i)\left[\sigma^2_{\hat{\alpha}_2} + i^2\alpha_1^2\sigma^2_{\hat{\alpha}_2} - 2\alpha_1 i\sigma_{\hat{\alpha}_1\hat{\alpha}_2}\right] \qquad (31)$$

ii. The values $\sigma^2_{\hat{\alpha}_1}, \sigma^2_{\hat{\alpha}_2}$ and $\sigma_{\hat{\alpha}_1\hat{\alpha}_2}$ are elements of the matrix

$$\begin{bmatrix} \sigma^2_{\hat{\alpha}_1} & \sigma_{\hat{\alpha}_1\hat{\alpha}_2} \\ \sigma_{\hat{\alpha}_1\hat{\alpha}_2} & \sigma^2_{\hat{\alpha}_2} \end{bmatrix} = \begin{bmatrix} \sigma^{11} & \sigma^{12} \\ \sigma^{12} & \sigma^{22} \end{bmatrix}^{-1} \qquad (32)$$

Assessment model for the overall probability of success in the clinical trial

Patients' response to treatment in clinical trials is classified as "success" or "failure". Models developed have been concerned in estimating the probability of success (failure) at each stage for clinical trials conducted in stages. We are to assess the overall probability of success at the end of each stage of the clinical trial conducted in k stages. At the first stage of the trial,$\tilde{R}_1$ the estimate for patient response is given by

$$\tilde{R}_1 = r_i = \frac{X_i}{n_i} \qquad (33)$$

Where, n_i = patients under stage i, X_i = patients are those who respond for stage i, $i = 1, 2, .., k$

The overall assessed probability of patient response after each stage the model is

$$\hat{R}_i = \widehat{\alpha_i^0}\, r_i + \left(1 - \widehat{\alpha_i^0}\right)\hat{R}_{i-1} \quad (34)$$

Where, r_i proportion of patient respond at the ìths stage

$\widehat{\alpha_i^0}$ is the smoothing constants of each stage

Using the empirical approach or method discussed by Gross [1971b] in determining the smoothing constant, we make the following assumptions,

- $\hat{R}_i$ is an unbiased estimator of R_i, where R_i is the theoretical assessment of the overall probability of success after the i^{th} trial, $i = 1, 2, .., k$
- $P_i = \epsilon(r_i)$, thus P_i is the probability that a patient on study during the i^{th} stage responds.
- All patients within a stage have the same independent probability of response. Furthermore, the stages are independent.

The criterion for the choice of each smoothing constant $\hat{\alpha}_i$ is to minimise the variance $\hat{\sigma}_i^{-2}$ of its associated value$\hat{R}_i$by assumption 1 through 3.

$$\hat{\alpha}_i^0 = \frac{\hat{\sigma}_i^{-2}}{\sum_{j=1}^{i} \sigma_i^{-2}} \quad (35)$$

$$\hat{\sigma}_i^2 = \frac{r_i(1-r_i)}{n_i - 1} \quad (36)$$

Hence

$$\hat{\sigma}_i^{-2} = \frac{n_i - 1}{r_i(1-r_i)} \quad (37)$$

$\hat{\boldsymbol{\sigma}}_i^{-2}$= inverse estimated variance for each stage

$\hat{\alpha}_i^{\mathbf{0}}$= smoothing constant for each stage

RESULTS AND DISCUSSION

Introduction

This chapter presents the details of the analysis and results of the research. This consists of presentation of data collected, numerical analysis (least square estimates and maximum likelihood estimates), confidence region and interval at each five stages of the of the clinical trial, assessment of the overall probability of success in the clinical trial and discussion of the results.

Presentation of data collected

Analysis on this study began with the data collected from the Akuse Government Hospital. The data collected was a clinical trial which was conducted in five (5) stages. The clinical trial was conducted with the aim of improving the probability that patients suffering from typhoid fever will be cured after receiving treatment. The data, which was made up of ten patients in each stage of the clinical trial adding up to fifty (50)

patients. Out of the fifty patients, twenty two (22) were male and twenty eight (28) were females.

The five staged clinical trial, has in each stage a total of ten patients who underwent the trials. In each stage of the clinical trial some of patients were unable to be cured of the disease, others did. The patients, who after two weeks, are supposed to be cured of the disease, were grouped into healed and not healed. That is, those patients that were cured of the disease were grouped under healed and those that were not cured, placed under not healed. Stage one of the clinical trial had six patients healed and four patients not healed. Stage two and stage three had seven patients healed and three patients not healed each. Stage four had eight patients being healed and two patients not healed. Stage five had nine patients out of ten healed.

The proportion of patients being cured at each stage of the clinical trial is derived. This is done by dividing the total number of patients that were healed in the stage by the total number of patients in that stage of the clinical trial. This proportion of patients also depicts the survival probabilities of each stage. The table below shows the details of the data collected.

Table 1: Stage by stage clinical trial with survival probability of each stage

Stages	Total number of patients	Number of males	Number of females	Number of patients healed	Number of patients not healed	Survival probability
1	10	4	6	6	4	0.6
2	10	3	7	7	3	0.7
3	10	5	5	7	3	0.7
4	10	6	4	8	2	0.8
	10	4	6	9	1	0.9

Table 1 shows an improvement in the number of patients that are healed from stage-to-stage. Plotting the survival probabilities of each stage aids in determining the exponential model used in the analysis of the data. The graph in Fig 2 shows the graphical display of the survival probabilities.

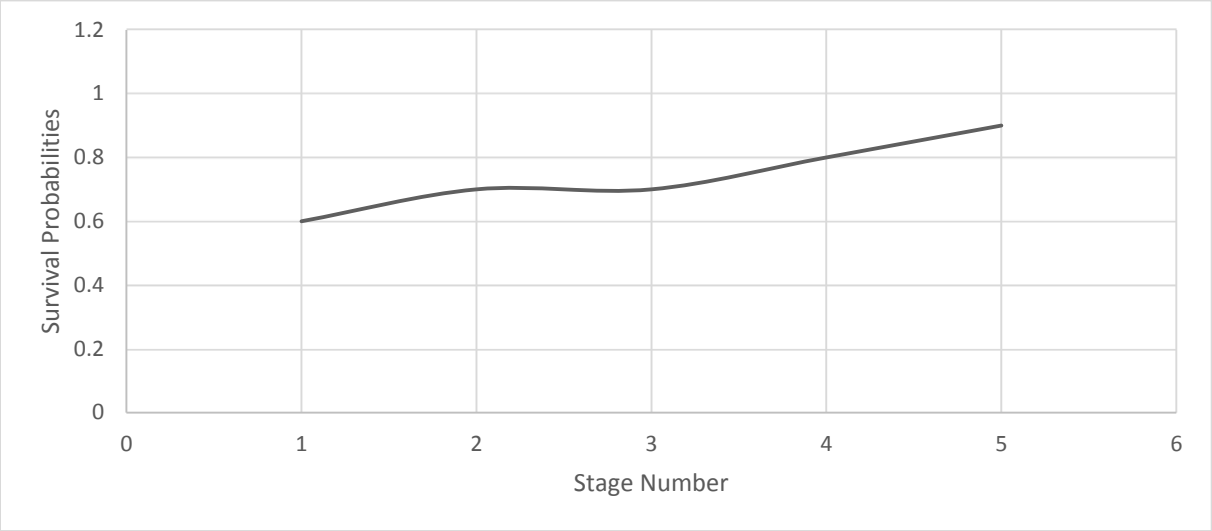


Fig 2: Graph of Survival probabilities as a function of the stages (i) for typhoid fever remission
X-axis = stages, Y-axis = survival probabilities

Fig 2 shows the survival probabilities having a slower growth in the early stages which sustains itself in the later stages. This determines the use of the exponential model in the analysis of the data. The exponential model is used when at the early stages of the clinical trial the survival probabilities show a slow growth and later sustains itself.

Numerical analysis
Two methods are commonly used in the estimation of $\alpha_1$and α_2. These are the maximum likelihood and least squares. Maximum likelihood estimators are generally preferred to least squares estimators because of the desirability of the large sample properties of maximum likelihood estimators. On the other hand, least square estimators are often obtainable in closed form and are good first approximation.

Least square estimates
The exponential model as given in equation (19), computes $\hat{\alpha}_1$and $\hat{\alpha}_2$, the maximum likelihood estimates of α_1 and α_2 respectively, where
α_1= the growth in the healing process (survivability growth)
α_2= the drug intervention (reliability of the typhoid drugs)

To this end, we obtained the initial estimates $\hat{\alpha}_{10}$ and $\hat{\alpha}_{20}$ by least square equations (17) and (18). The estimates $\hat{\alpha}_{10}$ and $\hat{\alpha}_{20}$ were obtained from the layout in Table 2.

Table 2: Least square computation layout

Stages i	Number of cured patients x_i	$\frac{n_i - x_i}{n_i + 1}$	$z_i = log_e \frac{n_i - x_i}{n_i + 1}$	iz_i
1	6	4/11	-1.01160	-1.01160
2	7	3/11	-1.29928	-2.59856
3	7	3/11	-1.29928	-3.89784
4	8	2/11	-1.70475	-6.81899
5	9	1/11	-2.39790	-11.98948
Totals			**-7.71281**	**-26.31647**

From Table 2 and equations (17) and (18), it follows that our initial estimates $\hat{\alpha}_{10}$ was 0.55480 and $\hat{\alpha}_{20}$ was 0.317805. These initial estimates are our least square estimates.

Maximum likelihood estimates

The initial estimates were used in the computation for the Newton-Raphson procedure. This procedure was used to obtain the maximum likelihood estimates of $\hat{\alpha}_1$and $\hat{\alpha}_2$. By the Newton-Raphson procedure, the maximum likelihood estimates was obtained by going through series of iterations for the Newton-Raphson procedure to converge at an estimate.

To facilitate the computation of the Newton-Raphson procedure, Table 3 is given. In this table we let$\hat{g}_{10i}$, $\hat{g}_{20i}$, $\widehat{-f}_{110i}$, $\hat{f}_{120i}$ and $\widehat{-f}_{220i}$ be, respectively,

$$-\frac{x_i}{[exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i) - \hat{\boldsymbol{\alpha}}_{\mathbf{10}}]} + \frac{\sum_{i=1}^{k}(n_i - x_i)}{\hat{\boldsymbol{\alpha}}_{\mathbf{10}}},$$

$$\frac{\hat{\boldsymbol{\alpha}}_{\mathbf{10}} i x_i}{[exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i) - \hat{\boldsymbol{\alpha}}_{\mathbf{10}}]} - i(n_i - x_i),$$

$$-\left(\frac{x_i}{[exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i) - \hat{\boldsymbol{\alpha}}_{\mathbf{10}}]^2} + \frac{n_i - x_i}{\hat{\boldsymbol{\alpha}}_{10}^2}\right),$$

$$\frac{i x_i exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i)}{[exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i) - \hat{\boldsymbol{\alpha}}_{\mathbf{10}}]^2},$$

and

$$-\hat{\boldsymbol{\alpha}}_{\mathbf{10}} \frac{i^2 x_i exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i)}{[exp(\hat{\boldsymbol{\alpha}}_{\mathbf{20}} i) - \hat{\boldsymbol{\alpha}}_{\mathbf{10}}]^2}.$$

For the first iteration we used the initial estimates in the computation.

Table 3: First iteration

Stages i	$\hat{g}_{10i}$	$\hat{g}_{20i}$	$\widehat{-f}_{110i}$	$\hat{f}_{120i}$	$\widehat{-f}_{220i}$
1	-0.1134454	0.06293951	-21.933657	12.28224	-6.814186
2	0.1575125	-0.17477582	-13.683755	14.86847	-16.498054
3	1.9755692	-3.28813741	-11.428942	13.09562	-21.796356
4	0.9474480	-2.10257666	-7.380420	12.58884	-27.937144
5	-0.2692907	0.74701253	-3.725733	11.68164	-32.404857
Total	2.697794	-4.755538	-58.15251	64.5168	-105.4506

The values $\hat{\alpha}_{11}$ and $\hat{\alpha}_{21}$were then found by solving the matrix equation

$$\begin{pmatrix} \hat{\boldsymbol{\alpha}}_{\mathbf{11}} \\ \hat{\boldsymbol{\alpha}}_{\mathbf{21}} \end{pmatrix} = \begin{pmatrix} \mathbf{0.5548} \\ \mathbf{0.317805} \end{pmatrix} - \begin{bmatrix} -58.15251 & 64.5168 \\ 64.5168 & -105.4506 \end{bmatrix}^{-1} \begin{pmatrix} 2.697794 \\ -4.755538 \end{pmatrix}.$$

Solving the matrix $\hat{\alpha}_{11} = 0.5435$and $\hat{\alpha}_{21} = 0.2658$. $\hat{\alpha}_{11}$and$\hat{\alpha}_{21}$ were used in the second iteration. The iteration is continued since the Newton-raphson procedure did not converge to maximum likelihood estimates.

Table 4: Second iteration

Stages i	$\hat{g}_{11i}$	$\hat{g}_{21i}$	$\widehat{-f}_{111i}$	$\hat{f}_{121i}$	$\widehat{-f}_{221i}$
1	-0.5249250	0.2852968	-23.902550	13.51596	-7.345925
2	-0.5243284	0.5699450	-15.374736	17.76099	-19.306201
3	1.3438211	-2.1911004	-12.647220	16.58983	-27.049715
4	0.2786689	-0.6058263	-8.216665	16.74835	-36.410921
5	-0.9432078	2.5631673	-4.245978	16.25448	-44.171559
total	-0.3699712	0.6214824	-64.38715	80.86962	-134.2843

The values $\hat{\boldsymbol{\alpha}}_{\mathbf{12}}$and $\hat{\boldsymbol{\alpha}}_{\mathbf{22}}$were then found by solving the matrix equation

$$\begin{pmatrix}\hat{\alpha}_{12}\\ \hat{\alpha}_{22}\end{pmatrix} = \begin{pmatrix}0.5435\\ 0.2658\end{pmatrix} - \begin{bmatrix}-64.38715 & 80.86962\\ 80.86962 & 134.2843\end{bmatrix}^{-1}\begin{pmatrix}-0.3699712\\ 0.6214824\end{pmatrix}.$$

Solving the matrix $\hat{\alpha}_{12} = 0.5438$and $\hat{\alpha}_{22} = 0.2706$. $\hat{\alpha}_{12}$and$\hat{\alpha}_{22}$ were used in the third iteration. The iteration was continued since there was no convergence of the maximum likelihood estimates.

Table 5: Third iteration

Stages i	$\hat{g}_{12i}$	$\hat{g}_{22i}$	$\widehat{-f}_{112i}$	$\hat{f}_{122i}$	$\widehat{-f}_{222i}$
1	-0.4675435	0.2542502	-23.726761	13.37016	-7.270691
2	-0.4444299	0.4833620	-15.221282	17.44353	-18.971579
3	1.4187522	-2.3145523	-12.543850	16.20778	-26.441373
4	0.3555019	-0.7732877	-8.142917	16.29047	-35.435021
5	-0.8676804	2.3592230	-4.195555	15.74612	-42.813689
Total	-0.005399756	0.008995103	-63.83036	79.05804	-130.9324

From Table 5 $\hat{\alpha}_{13}$ and $\hat{\alpha}_{23}$were found by solving the matrix

$$\begin{pmatrix}\hat{\boldsymbol{\alpha}}_{\mathbf{13}}\\ \hat{\boldsymbol{\alpha}}_{\mathbf{23}}\end{pmatrix} = \begin{pmatrix}0.5438\\ 0.2706\end{pmatrix} - \begin{bmatrix}-63.83036 & 79.05804\\ 79.05804 & -130.9324\end{bmatrix}^{-1}\begin{pmatrix}-0.005399756\\ 0.008995103\end{pmatrix}.$$

Solving the matrix,$\hat{\alpha}_{13} = 0.5438$ and $\hat{\alpha}_{23} = 0.2707$ and were then used in the fourth iteration to verify the convergence of the maximum likelihood estimates.

Table 6: Fourth iteration

Stages i	$\hat{g}_{13i}$	$\hat{g}_{23i}$	$\widehat{-f}_{113i}$	$\hat{f}_{123i}$	$\widehat{-f}_{223i}$
1	-0.4662067	0.2535232	-23.723275	13.36692	-7.268933
2	-0.4426859	0.4814652	-15.218312	17.43681	-18.964272
3	1.4203726	-2.3171958	-12.541953	16.19982	-26.428394
4	0.3571305	-0.7768302	-8.141565	16.28101	-35.414452
5	-0.8661063	2.3549430	-4.194608	15.73567	-42.785292
total	0.002504157	-0.004094581	-63.81971	79.02024	-130.8613

From Table 6, $\hat{\alpha}_{14}$ and $\hat{\alpha}_{24}$ was found by solving the matrix

$$\begin{pmatrix}\hat{\alpha}_{14}\\ \hat{\alpha}_{24}\end{pmatrix} = \begin{pmatrix}0.5438\\ 0.2707\end{pmatrix} - \begin{bmatrix}-63.81971 & 79.02024\\ 79.02024 & -130.8613\end{bmatrix}^{-1}\begin{pmatrix}-0.004094581\\ -0.004094581\end{pmatrix}.$$

Solving the matrix, $\hat{\alpha}_{14} = 0.5438$and $\hat{\alpha}_{24} = 0.2707$. The iteration was stopped since the Newton-Raphson procedure converged to the maximum likelihood estimates. Table 7 shows the results at each step of the iteration. The third iteration was chosen since further iterations gave the requisite maximum likelihood estimates and does not change the result.

Table 7: Values of $\hat{\alpha}_{1j}$ and $\hat{\alpha}_{2j}$ at each iteration of the Newton-Raphson procedure

Iteration j	$\hat{\alpha}_{1j}$	$\hat{\alpha}_{2j}$
0	0.5548	0.317805
1	0.5435	0.2658
2	0.5438	0.2706
3	0.5438	0.2707
4	0.5438	0.2707

Table 7 shows clearly that after the second iteration, the estimates begin to become the same, that is, they converge.

It is intrusive to compare the observed and expected relief probabilities at each stage using both least squares and maximum likelihood estimates. These comparisons appear in Table 4.10.

Table 8: Expected and observed relief probabilities for typhoid fever patients at each stage when the clinical trial is conducted

Stages i	Observed Probability	Expected Maximum Likelihood Estimate probability	Expected Least Squares likelihood Estimate probability
1	0.6	0.5851647	0.5962472
2	0.7	0.6835448	0.7061711
3	0.7	0.7585937	0.7861676
4	0.8	0.8158443	0.8443846
5	0.9	0.8595177	0.8867517

Table 8 indicates the growth in survivability at each stage of the clinical trial. The table also indicates that the least square estimates tend to overestimate and the maximum

likelihood estimates tend to underestimate the observed probabilities of relief at each stage. Thus the least square estimates are somewhat optimistic whereas the maximum likelihood estimates are somewhat conservative.

Confidence region and interval for the probability of relief

We obtained an appropriate (1-α) 100 percent lower confidence limit $p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2)$ for the model by means of (25). Thus the values of $\sigma^2_{\hat{\alpha}_1}, \sigma^2_{\hat{\alpha}_2}$ and $\sigma_{\hat{\alpha}_1\hat{\alpha}_2}$ was obtained by inverting the matrix of $\sigma^{11}, \sigma^{12} and \sigma^{22}$ as by equations (27), (28) and (29), respectively. Thus, using the maximum likelihood estimates, we found$\sigma^{11} = 63.5497$, $\sigma^{12} = -79.2504$and $\sigma^{22} = 131.2249$. Thus we see that

$$\begin{bmatrix} \sigma^2_{\hat{\alpha}_1} & \sigma_{\hat{\alpha}_1\hat{\alpha}_2} \\ \sigma_{\hat{\alpha}_1\hat{\alpha}_2} & \sigma^2_{\hat{\alpha}_2} \end{bmatrix} = \begin{bmatrix} 63.5497 & -79.2504 \\ -79.2504 & 131.2249 \end{bmatrix}^{-1} = \begin{bmatrix} 0.0637 & 0.0385 \\ 0.0385 & 0.0309 \end{bmatrix}.$$

By through equations (30) through to (32), we found that

$$p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2) = (1 - 0.5438\exp(-0.2707i)) - (1.645)\sqrt{\exp(-0.5414i)[0.0637 + 0.01138516i^2 - 0.01680342i]}$$

is a 95 percent lower confidence limit for $p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2)$, $i = 1,2, \ldots ,6$, noting that 1.645 is the upper 95th percentage point of the standard normal distribution.

Table 9: Ninty-five (95) percent lower confidence limits for the probability of relief from typhoid at each of the five clinical trial, plus predicted sixth stage lower limit

Stages i	$p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2)$
1	0.3340049
2	0.5299582
3	0.6604890
4	0.7432562
5	0.7957258
6	0.8324348

Table 9 indicates the values of $p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2)$ for the exponential model at each of the five stages of the clinical trial and in addition, the predicted value $p_{L6}(\hat{\alpha}_1, \hat{\alpha}_2)$ with confidence 0.95.

Assessing the overall probability of success in the clinical trials

Assessing the overall probability of success at each stage of the clinical trial, the values of $\hat{\sigma}_i^{-2}$, α_i^0 and $\hat{R}_i$ was found.

Table 10: Assessed overall survival probabilities at each stage of the clinical trial

Stage i	r_i	$\hat{\sigma}_i^{-2}$	$\hat{\alpha}_i^0$	$\hat{R}_i$
1	0.6	37.5	-	0.600
2	0.7	42.86	0.533	0.653
3	0.7	42.86	0.348	0.669
4	0.8	56.25	0.313	0.710
5	0.9	100.00	0.358	0.778

Table 10 contains the overall assessed probability of patients' response after each stage as well as the inverse estimated variance and smoothing constant for each stage. The estimated variances were found to be 37.5, 42.86, 42.86, 56.25 and 100.00. Our smoothing constants were found to minimize the variances of its associated value$\hat{R}_i$. The smoothing constant were 0.533, 0.348, 0.313 and 0.358. $\hat{R}_i$was our overall remission probabilities from typhoid fever at each stage.

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

Typhoid fever is one of the top 10 out-patient diseases in Ghana according to the Ghana health service.It is rated among the top killer diseases in Ghana. The research began with information gathered from the Akuse Government Hospital depicting a rise in the number of typhoid fever cases recorded from 2011, 2012 to 2013. The figures recorded by the hospital were 322, 772 and 1000. These indicate an increase from year to year. The increase was amounted to the fact that the residents in the Akuse Township had bad hygienic practices which has contributed to the spread of the disease in the vicinity.

In this regard, the research was interested in assessing the survivability growth and reliability of typhoid fever drugs in Akuse.

The main objectives of the research was to assess the survivability growth and reliability of typhoid fever drugs and also the overall probability of survival in each stage of the clinical trial conducted by the hospital. The methodology employed to assess the survivability growth was to find least squares and maximum likelihood estimates of the exponential model chosen to analyze the data collected from the hospital. The exponential model was chosen after the data collected from the hospital was plotted on a graph. The graph showed a slow survivability growth in the early stages which sustained itself in the later stages of the clinical trial.

The maximum likelihood estimates and the least squares estimates was used to find expected maximum likelihood estimate probabilities and expected maximum likelihood probability estimates, respectively, which was compared with the observed probability estimates. The confidence interval and region for the probability of relief at each stage was also calculated. The overall probability of success at each stage of the clinical trial was calculated for by finding the inverse estimated variance and smoothing constants for each stage. These in turn aid in assessing the overall probability of success in each stage of the clinical trial.

The Least squares estimates were found to be the initial estimates of the maximum likelihood estimates. These initialestimates $\hat{\alpha}_{10}$ was 0.55480 and $\hat{\alpha}_{20}$ was 0.317805. The initial estimates was used in the computation for the Newton-Raphson procedure for the calculation of the maximum likelihood estimates. By the Newton-Raphson procedure, the maximum likelihood estimates was obtained by going through series of iterations for the Newton-Raphson procedure to converge at an estimate. After the second iteration, the Newton-Raphson procedure converged at $\hat{\alpha}_{12} = 0.5438$and $\hat{\alpha}_{22} = 0.2706$. The third iteration was chosen since further iterations gave the requisite maximum likelihood estimates and does not change the result. The observed and expected relief probabilities at each stage using both least squares and maximum likelihood estimates was compared.

A 95 percent lower confidence limit for $p_{L_i}(\hat{\alpha}_1, \hat{\alpha}_2)$, $i = 1,2,\ldots,6$, noting that 1.645 is the upper 95th percentage point of the standard normal distribution was constructed. Each stage with its lower limit was found to be 1=0.3340049, 2=0.5299582, 3=0.6604890, 4=0.7432562, 5=0.7957258 and 6=0.8324348. A predicted value $p_{L6}(\hat{\alpha}_1, \hat{\alpha}_2)$ with confidence 0.95 was also calculated for.

Finally, in finding the assessed overall probability of success, the smoothing constants were calculated for and was used in assessing the overall probability of success. The overall remission probabilities for stage 1 through to five were 0.600, 0.653, 0.669, 0.710 and 0.778, respectively.

CONCLUSION

From the analysis made and estimates derived in the Newton-Raphson procedure, we conclude that since $\hat{\alpha}_2 > 0$, the drug is reliable and very useful in the healing process of typhoid fever.

On whether the healing process is improving, conclusion was made on the observed survival probabilities derived from the data collected from the hospital. The observed probabilities showed an increase in the number of survivors at each stage of the clinical trial. This depicts improvement in the healing process. This is further confirmed by the expected least squares estimates probabilities and maximum likelihood estimates probabilities derived for each stage which show increasing probabilities from stage to stage. Noting that $p_i$is a function of α_1 and α_2, and also since α_2 is increasing that is, the drug reliability increase from stage to stage, this in turn affects the survivability growth and hence we conclude that there is improvement in the healing process.

Considering the probability values derived in the assessed overall survival probabilities, we conclude that a patient that faces treatment in stage one will have a 0.6 chance of survival, whereas stages two and three have 0.653 and 0.669 chances of survival respectively. Stages four and five had their overall survival probabilities to be above 0.7 and hence a patient chance of being cured of typhoid fever in that stage is 0.7 which is a very high chance of success.

RECOMMENDATION

From the conclusions theseare the recommendations we came out with.

- It is obvious that typhoid fever has very high chances of being cured and hence people affected with the disease are advised to visit the hospital to seek treatment.
- The Akuse Government Hospital in their endeavor to increase the curing rate of typhoid fever in the township should encourage patients to take drugs prescribed to them.
- The Ghana Health Service should engage in more clinical trials to improve upon the overall survival probabilities of patients
- Future assessments and or researchers are encouraged to collect primary data for their research.

REFERENCES

Alan J. Gross, Virginia A. Clarke, (1975), *Survival Distribution and Reliability Applications in the Biomedical Sciences*. New York: John Miley and Sons

Balley and Scott's (1973) *Diagnostic Microbiology*, 9th Ed. Pasadena City College.

Encarta Interactive World Atlas (2007), Freshwater Contamination

Giannella R.A. (1996) *"Salmonella" Baron's Medical Microbiology* 4th Ed., University of Texas Medical Branch, ISBN0-9-631172-1-1.

Lasing .M. Prescott, John .P. Harley and Donald .A. Klien (2005), *Microbiology*, 3rd Ed. McGraw-Hill Company, Inc.

Mathlab Version R2007b

Nicolson Stuart (2008) *"Typhoid Fever Led to the Fall of Athens"*, BBC news, http://www.bbc.co.uk/2/hi/uk_news/scotland/7471198.com.

R Statistical Software version i386 3.0.2

Retief F.P., Cilliers L., (1998), S. Afr Med J.; Pp 88 (1): 50-3.

Ryan J. Kenneth, C. Ray, (2004), *Sherris Medical Microbiology: An Introduction to Infectious Diseases*, 4th ed. McGraw Hill, ISBN 0838585299.

Smith A. L. (1985), *Principle Of Microbiology*, 10th ed., Elsevier, Pp 945..

Typhoid Vaccines: World Health Organization position paper, weekly Epidemiology Rec 83 (6): 45-49, February 2008, PMID 18260212, http://www.who.int/entity/wer/2008/wer8305.pdf .

World Health Organization (2008) *Typhoid Fever Fact Sheet*, (www.WHO.int). Centre for Disease Control and Prevention, Typhoid fever disease information.
http://www.wikipedia.org/wiki/typhoid_mary